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ABSTRACT

The human genome project started in 1989 with the collaboration of the National Institutes of Health (NIH) and the U.S. Department of Energy (DOE). This document aims to develop an understanding among students of the human genome project and relevant issues. Topics include the science and technology of the human genome project, and the ethical and public policy dimensions of the project. Four activities are presented in the document: (1) "DNA Sequences"; (2) "Do Our Genes Determine Our Future?"; (3) "The Case of Nathaniel Wu"; and (4) "Public Policy: Genetics and Alcoholism". Four chapters of instructional information on DNA and appropriate activities related to the chapters are also presented. Chapters include: (1) "The Hereditary Molecule"; (2) "Passing Traits from One Generation to the Next"; (3) "How Genes and the Environment Influence Our Health"; and (4) "Controlling Our Genetic Futures." Appendices include Timeline, Helpful Hints, and Some Frequently Used Suppliers. (Contains 39 references.) (YDS)

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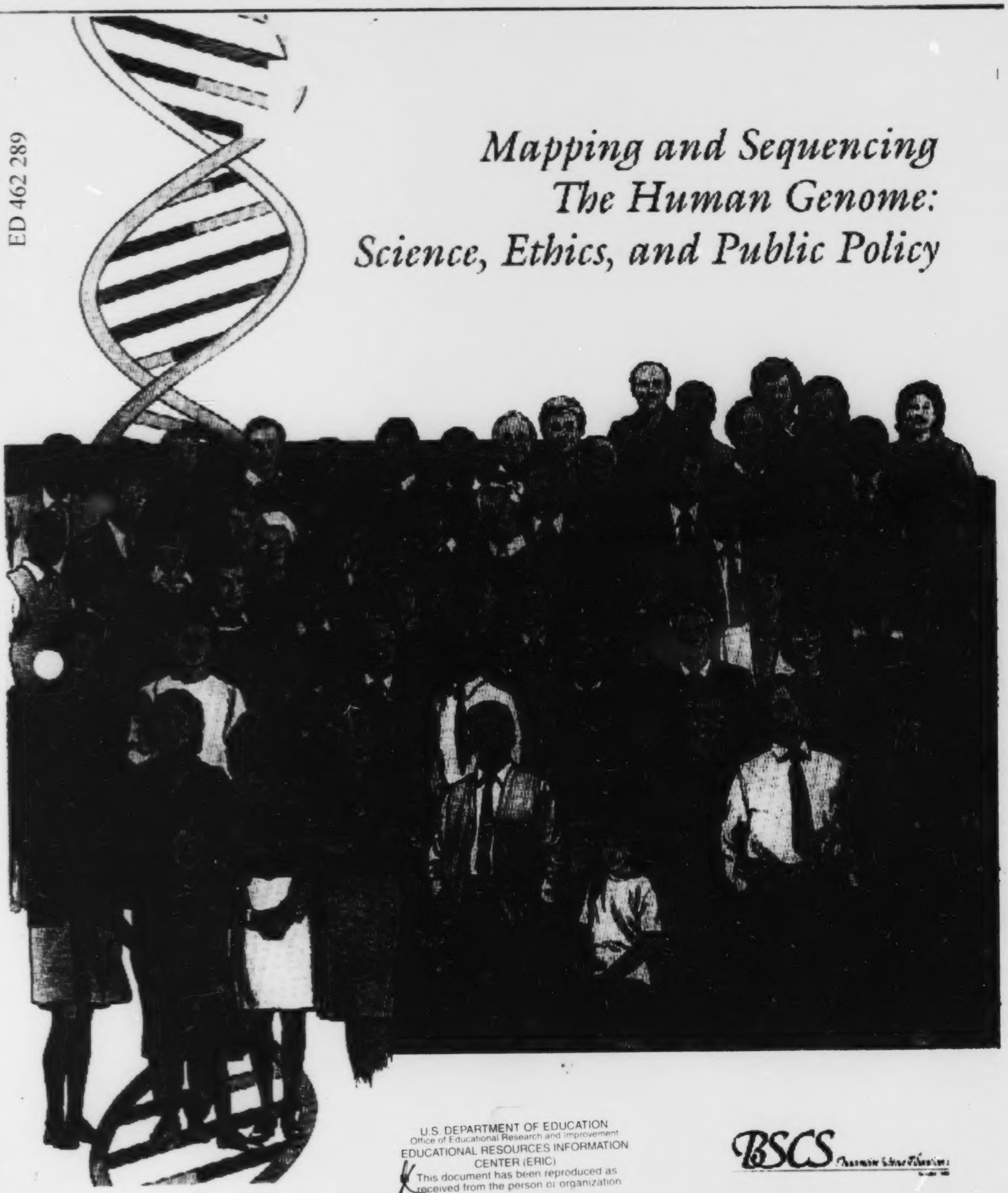
Mapping and Sequencing The Human Genome: Science, Ethics, and Public Policy



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Foreword

During the summer of 1990, the popular press worldwide carried startling news about research into the genetics of cystic fibrosis (CF), a serious disorder of the lungs, pancreas, and other organs that affects approximately 50,000 young people in the United States. Building upon previous work that mapped the gene for CF to chromosome 7, molecular biologists isolated the gene and determined its sequence.

This landmark work highlights the potential benefits and pitfalls of the Human Genome Project (HGP), the $3 billion, 15-year enterprise that seeks to map and sequence all of the estimated 100,000 human genes. The HGP will use and expand many of the scientific insights and technological innovations that enabled Francis Collins of the University of Michigan and Lap-Chee Tsui of the Toronto Hospital for Sick Children to isolate and sequence the CF gene. These insights and technologies— and the implications for individuals and society—demonstrate the intimate relationship between science, technology, and society (Figure **A**).

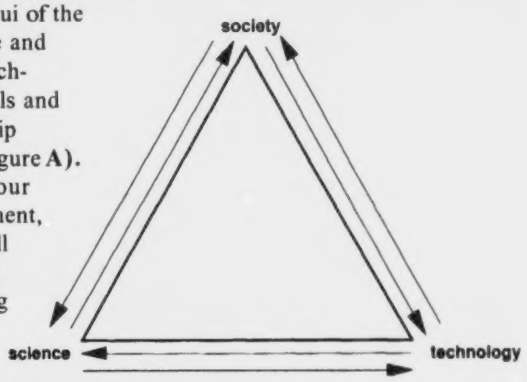

Figure A The interrelationships between science, technology, and society

The HGP has the potential to increase our understanding of human variation, development, gene regulation, and human evolution. It will advance the practice of medicine by opening doors to new ways of diagnosing and treating inherited disorders. The HGP also will increase the demand for individuals trained in genetics, medical genetics, genetic counseling, and other health fields. The development of new technologies, especially those to help manage the enormous amounts of data the HGP will generate, will have applications far beyond the project itself.

The greater medical and scientific knowledge made possible by the HGP also will lead to potential difficulties such as the ability to diagnosis genetic disorders before we have the means to treat them, the temptation to restrict reproduction to those with desirable traits, and unjustified discrimination based on an individual's genetic profile. The HGP will continue to raise such ethical and public policy issues of uncertainty about (1)the relative and variable contributions of genes and the environment to complex human traits, (2) the ability of tests to predict the onset or severity of disorders that have a genetic component, (3)our ability to regulate fairly the commercial applications of the HGP, and **(4)**our ability to protect the interests of individuals and institutions through new public policies when our understanding of the science is incomplete and further complicated by the rapid development of technology and by long-standing public fears and misconceptions about genetics.

The progress scientists have made in the molecular pathology of CF illustrates how such uncertainties can affect individuals, families, and society. Research on the molecular genetics of CF is important to society because CF is a debilitating, chronic disorder. CF is one of the most common inherited disorders for Caucasians—1 in every 500 individuals is heterozygous for the CF allele. Currently, individuals who have CF have a life expectancy of approximately 29 to 30 years 40 years less than the average life expectancy of the American population. Although treatments for CF are improving continually and research on gene therapy is in preliminary stages, families confront significant responsibilities for the long-term care of children with CF. Parents of children born with the disorder face difficult decisions about the recurrence of the disorder in subsequent pregnancies, and society expends considerable health-care resources on the treatment of children and adults with CF.

The prevalence of the CF gene mutations in the population and the ability to detect these mutations with increasing sensitivity have led *some* researchers to recommend population-based screening to detect the carriers of the CF allele. Such a program, which could affect millions of couples of child-bearing age, is a matter of considerable controversy. Most researchers think that large-scale screening programs should be proceeded by smaller pilot programs. Other researchers are completely opposed to population screening for CF because the tests cannot detect all of the mutations. In addition, if prenatal genetic testing for CF were to become widespread, difficult questions would arise about the accuracy of the testing and about the cost of testing all pregnancies as opposed to testing only those pregnancies at risk because of family history. These events also could intensify the ongoing debate about abortion. Obviously, these societal concerns could affect the direction of genetic science and the subsequent development of technology, thus completing the feedback loop among science, technology, and society.

Interactions among genetic science, technology, and society often generate controversy that affects the interests ofindividuals, institutions, and society. The interests of an individual may be advanced by learning that, despite a positive family history, he or she has not inherited a gene for a disorder. Conversely, an individual's interests may be impaired by learning that he or she has a genetic predisposition to a lethal, incurable disease. The interests of institutions may be promoted by medical applications that could save many lives or improve the quality of life for many people. The interests of institutions may be impaired, however, by threats to the rights of privacy posed by the inability to protect completely the large, electronic data bases of genetic information that the HGP will produce. In addition, the interests of individuals, institutions, and society may be affected in ways we cannot identify at present because of the inherent uncertainty of the rate or direction of progress in genetic science and technology.

Your students will live their adult lives in an era influenced by the HGP, and it is important that they have some idea of its objectives, its scope, its inherent assumptions, and its potential implications. Our intent is to provide your students with the knowledge and skills required to understand the HGP at a basic level and to analyze the relevant issues in a manner that fosters informed, respectful debate and sound decision making. We hope you will enjoy using these materials with your students, and we welcome your feedback.

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History, Organization, and Funding of the Human Genome Project

Advances in several fields made it possible to conceive of a human genome project by 1985. The principal advances included the ability of scientists to isolate and manipulate DNA, which dates from the mid-1970s, and the construction of parts of the human genome map by several research groups in the 1980s. By 1990, the National Institutes of Health (NIH) and the U.S. Department of Energy (DOE), the federal agencies primarily responsible for the organization and funding of the Human Genome Project (HGP) in the U.S., had submitted a five-year, joint research plan to Congress.

The collaboration of NIH and DOE in planning the U.S. genome project began in 1989 and continued with the submission of their joint research proposal in 1990. The two agencies have established common working groups on mapping and informatics (data bases and computational analysis), as well as on the social, ethical, and legal implications of genome research. The agencies also sponsor many joint conferences and workshops and are working toward cross-referencing grant applications and funding decisions. The Howard Hughes Medical Institute (HHMI) has had a strong interest in human genetics for many years and supports several genetic data bases as well as some of this country's leading investigators in human genetics.

A number of Human Genome Program Centers throughout the country serve as focal points for coordinating genome research. The Human Genome Program Centers also provide data and materials to outside researchers who have an interest in the same genetic region. The major organizations involved in funding genome research projects are the National Institutes of Health, the Department of Energy, the National Science Foundation, and the Howard Hughes Medical Institute. Figure B shows the allocation of funds across various aspects of the project.

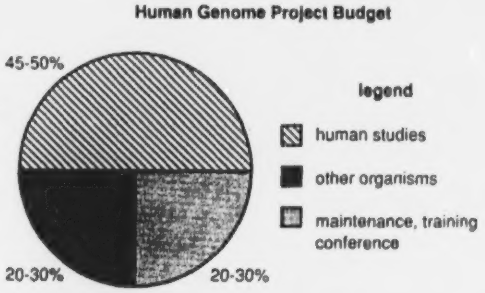

Figure B Human genome project budget

The Human Genome Organization (HUGO), formed in 1988, mediates international scientific collaboration. The United Nations Educational, Scientific, and Cultural Organization (UNESCO) promotes the continued involvement of developing nations in genome activities and also supports international conferences and exchanges. Genome programs have begun in Italy, the United Kingdom, France, the Commonwealth of Independent States, the European community, Japan, and Canada.

Section I
The Science and Technology of the Human Genome Project

THE CONTEXT OF THE HUMAN GENOME PROJECT

Human cells that have nuclei (except for eggs and sperm) normally contain 22 pairs of autosomal chromosomes, plus 2 sex chromosomes (XX or XY), and many mitochondria, each with a small, circular chromosome. The total genome for any individual includes all of these components. For the purposes of the Human Genome Project (HGP), however, *genome* means one each of the different chromosomes—22 autosomes, plus X, plus Y, plus a mitochondrial chromosome. The genome contains approximately 3 billion base pairs of DNA, which contain 50,000 to 100,000 genes—the basic units of heredity. Although two individuals, as well as the members of a chromosome pair, differ in details, the *basic* map is the same. The major "housekeeping" genes are in the same location for all individuals. Therefore, the HGP requires only one of the pair of chromosomes. The knowledge and technology that result from the HGP eventually may allow researchers to describe the total genome of an individual.

OBJECTIVES OF THE HUMAN GENOME PROJECT: MAPPING AND SEQUENCING

The HGP has two major objectives. The first is to develop detailed maps of the human genome and the genomes of several other well-studied organisms: bacterium, yeast, nematode, fruit fly, mouse, and *Arabidopsis thaliana*, a rapidly growing plant that has a small genome. As species evolve, some parts, including genes, are conserved, that is, they continue to exist. This is true on a molecular and genetic level as well as on the morphological level. Thus, by learning about the genes of an animal such as a mouse or a nematode, scientists can develop a deeper understanding of similar genes encountered in humans.

Another reason for sequencing the genomes of other organisms is that they contain less "junk DNA" than does the human genome. In other words, there is more genetic information per given sequence. This makes it easier for molecular biologists to locate functional sequences. Table 1 (on page 2) provides a comparison of sequence lengths in various structures and organisms. A major focus of the HGP is the development of sequencing schemes that are faster and more economical.

The second objective of the HGP is to determine the complete base sequences of these genomes. (The sequence for human mitochondrial DNA already has been determined.) Because a written record of the human genome sequences would require the equivalent of 200 telephone books of 1,000 pages each, one essential part of the project is the development of systems for electronic data base storage and management to han-

Table 1 Comparison of Known Sequences with Chromosome and Genome Sizes

Comparative Sequence	No. of Bases
Largest known continuous DNA sequences (yeast chromosome 3)	350 thousand
Escherichia coli (bacterium) genome	4.6 million
Largest yeast chromosome now mapped	5.8 million
Entire yeast genome	15 million
Smallest human chromosome (Y)	50 million
Largest human chromosome (1)	250 million
Entire human genome	3 billion

dle the tremendous amount of information the HGP will generate.

Geneticists use two types of maps to characterize the human genome: genetic linkage maps and physical maps. A *genetic linkage* map assigns a distance between markers (genes for inherited traits or known fragments of *DNA)* according to the frequency with which they are inherited together, that is, not separated by recombination during meiosis. In general, the closer the markers are physically, the more likely they will be inherited together. To be useful for tracing inheritance, markers on a genetic linkage map must vary between individuals. For example, a parent might have form *a* of a given marker on one chromosome of a pair and form *b* on the other, making it possible to determine which of the two forms was passed on to his or her child.

The second type of map is a *physical* map, in which researchers measure the actual physical distance between two points, such as between *DNA* markers, without regard to the frequency of recombination. The units of measurement usually are nucleotides. The base sequence of *DNA* is an extremely detailed physical map. Figure 1 compares these two types of maps.

HGP investigators use genetic linkage and physical maps of different levels depending on whether they wish to obtain an overall view of a large portion of *DNA*, such as a chromosome (usually a genetic linkage map), or a more detailed

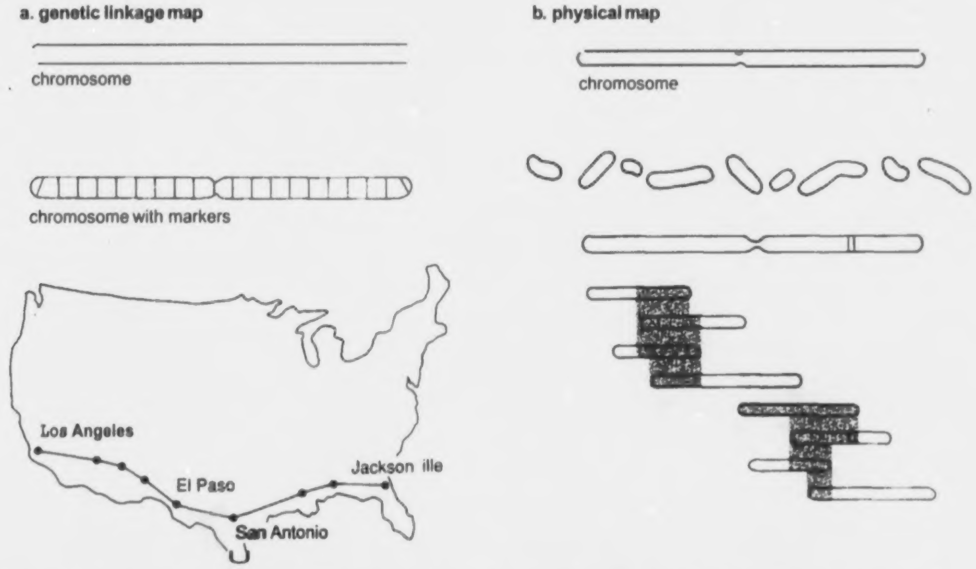

Figure 1 Genetic linkage maps of chromosomes (a) are similar to establishing the positions of towns on a road map. Interstate 10 runs from Florida to California. If there were no towns mapped along that highway, it would be difficult to locate a landmark between, for example, Jacksonville and Los Angeles. It is easier to locate a landmark if it is located between markers that are closer together, such as El Paso and San Antonio. Physical maps (b) are created by chemically cutting the chromosomes into pieces and reordering them the way they were on the chromosome. Because researchers will know where on the chromosome each piece came from, they can tell where any gene in that piece is located on the chromosome.

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view of a specific region, such as the area in which one expects to find a gene (usually a physical map). Figure 2 illustrates these levels.

Researchers involved in the HGP generally agree that preparation of detailed physical maps should be a priority and that sequencing the entire genome should occur only when advances in technology allow sequencing to be done at a reasonable cost. The goals set by the primary funding agencies for the first five-year period, ending in 1995, include the following:

- generating genetic linkage and physical maps that cover all the human chromosomes. The markers for the physical map should be approximately 100,000 base pairs apart;
- generating sets of overlapping cloned fragments of human DNA for large parts of the human genome;
- improving the current sequencing techniques to allow sequencing at 50 cents per base pair and the piloting of large-scale sequencing projects that use DNA from humans and other organisms; and
- developing software and data base systems to support large-scale mapping and sequencing pro;jects and developing tools for easy access to the information.

If the cost of sequencing can be reduced to a reasonable amount, the remainder of the HGP (an estimated 10 years) will focus on the sequencing of the human genome and that of other organisms.

TECHNIQUES FOR MAPPING GENES

During the last decade, research in molecular biology has shown that a gene is composed of a number of exons separated by introns and also includes neighboring noncoding regions that control synthesis and processing of messenger RNA (mRNA). An exon is a segment of DNA that is transcribed into RNA and translated into protein; an exon specifies the amino acid sequence of a portion of the complete polypeptide. An intron is a segment of DNA that is transcribed into precursor mRNA, but then is removed before the mRNA leaves the nucleus. As the HGP provides new information about exons and introns, however, this definition of a gene may change.

A number of techniques help scientists determine the location of a gene within the genome. These techniques, which vary in accuracy, may allow biologists to assign a gene to a particular chromosome. Other techniques may allow them to determine more precise locations, or to determine the base sequence.

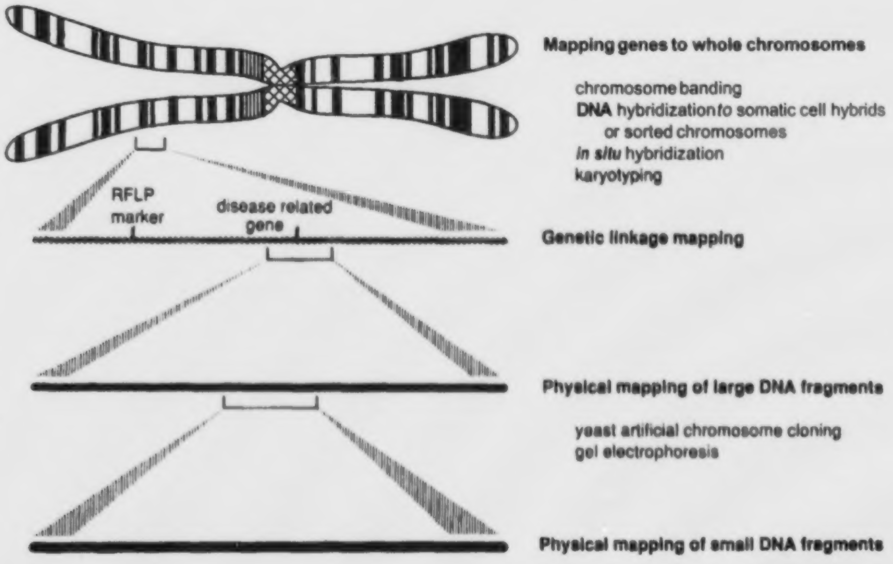
Mapping genes to whole chromosomes
chromosome banding
DNA hybridization to somatic cell hybrids or sorted chromosomes
in situ hybridization
karyotyping
RFLP marker
disease related gene
Genetic linkage mapping
Physical mapping of large DNA fragments
yeast artificial chromosome cloning
gel electrophoresis
Physical mapping of small DNA fragments

Figure 2 Mapping at different levels of resolution

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LOCALIZING GENES AND MARKERS ON CHROMOSOMES

Cytogenetics. Cytogenetic techniques allow researchers to see and examine individual chromosomes. Cytogeneticists use various staining methods to identify individual chromosomes, usually from the metaphase stage of mitosis. One method, called banding, shown in the karyotype Figure 3, makes dark bands appear in characteristic positions on different chromosomes. Banding is done by breaking open nuclei from dividing cells and fixing the chromosomes on a slide. Researchers then stain the chromosomes and examine and photograph them through a microscope. Analysis of the banding patterns permits researchers to identify individual chromosomes and to detect major chromosomal abnormalities.

Chromosomal rearrangements. On rare occasions, a genetic disorder results from a major chromosomal abnormality, for example, either a large (cytogenetically visible) deletion or a translocation — where one part of a chromosome breaks off and attaches to another chromosome. Patients with such chromosomal abnormalities are extremely valuable in the search for disease-related genes because the site of the chromosomal disruption corresponds to the gene location. Researchers cloned the gene for Duchenne Muscular Dystrophy (DMD,) for example, after investigating a few key DMD patients. One patient was a boy with DMD resulting from a cytogenetically visible deletion within his X chromosome. Further investigation of DNA from this patient led to the cloning of the DMD gene in 1987.

Cloning is one method scientists use to produce enough DNA for use as probes and other types of analysis. (The other method is the polymerase chain reaction—PCR—explained on page 8.) Cloning uses recombinant DNA technology to reproduce desired DNA fragments inside hosts such as bacteria, viruses, or yeast. The desired DNA fragment is removed from the chromosomes using restriction enzymes and is inserted into a vector (a carrier) such as a bacterial plasmid, which has been cleaved with the same restriction enzyme. This recombinant vector then is inserted into a host cell such as a bacterium, which will reproduce the desired DNA when the plasmid replicates.

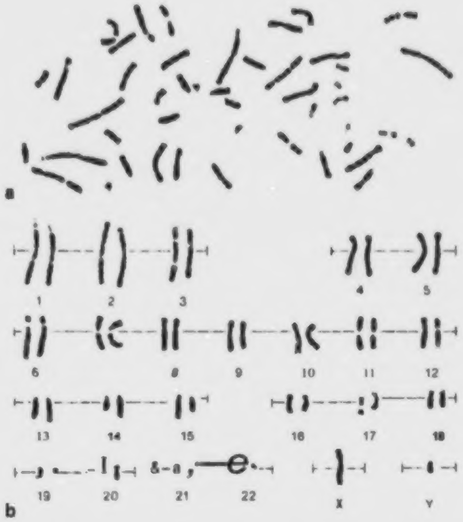

Figure 3 The karyotype of a male. The chromosomes from a dividing cell are stained (a), photographed, and the photograph is cut apart. The individual chromosomes then are arranged in a standard order of largest to smallest (b). The 22 pairs of autosomal chromosomes are arranged first and the two sex chromosomes usually are placed at the end.

***In situ* hybridization.** As the section on cytogenetics explains, geneticists can prepare cells in such a way that they can distinguish the chromosomes from one another. They then can use an isolated fragment of radioactively-tagged DNA from the gene under investigation as a probe. The probe hybridizes (sticks) to the chromosome that contains the complementary sequence and thus reveals the location of the gene from which the probe came. This technique is call *in situ* hybridization (*insitu* means in the original place) because the hybridization — the binding of the probe to the target sequence — occurs on the chromosomes themselves, rather than on DNA isolated from the chromosome, and the location of the hybridization is important.

Somatic-cell hybrids. Laboratory researchers can produce hybrids between cells from different organisms, for example, human and hamster cells. Initially these fused cells contain a complete set of chromosomes from the human and hamster parent cells. The hybrid cells grow and divide and lose chromosomes with each generation until they

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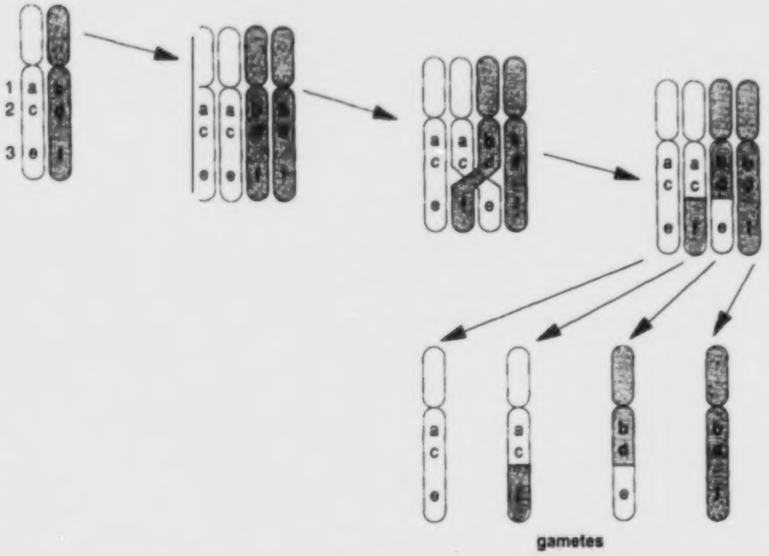

Figure 4 Crossing-over and recombination during meiosis. Crossing-over can occur at one or more points along adjacent chromosomes, leading to an exchange of **DNA.** Such recombination may cause alleles that previously were on the same chromosome to become separated. For example, markers 1, 2, and 3 each have two alleles (a and b, c and d, and e and f), and crossing-over occurs between markers 2 and 3.

finally stabilize. Human chromosomes are shed more easily from the hybrid cells than are hamster chromosomes. The hybrid cell lines usually end up containing 8 to 12 of the original 46 human chromosomes, in addition to the remaining hamster chromosomes. Researchers take a large set of somatic-cell hybrids and compare the presence or absence of a specific human chromosome with the presence or absence of a particular trait. If, for example, a particular detectable feature, such as resistance to a drug, is found only in a somatic-cell hybrid line that contains chromosome 5, it is likely that the gene responsible for this drug resistance is on human chromosome 5. Usually the gene under study does not express a phenotype that is recognized readily in the hybrid cell. In this case, a DNA fragment from another known gene is used as a probe in DNA that has been isolated from the various somatic-cell hybrids. Again, the appropriate chromosome is the one common to all the hybridizing cell lines. Researchers now use hybrid cell lines that contain single copies of human chromosomes 7, 16, 17, 19, X, and Y. The somatic-cell lines have made it much easier for researchers to create low-resolution physical maps of the genes on those chromosomes.

LOCALIZING GENES ASSOCIATED WITH DISEASE

X-linked traits. The first genes researchers assigned to specific human chromosomes are those that demonstrate X-linked inheritance. In 1911, E.B. Wilson reasoned that color blindness must be carried on the X chromosome because only males are affected and the disorder is transmitted through symptomless females. The assignment of other genes to the X chromosome, such as those for hemophilia and DMD, has been relatively straightforward because of a similar pattern of inheritance.

Genetic linkage analysis. Genetic linkage analysis allows geneticists to track a gene and determine its approximate location without cloning, or duplicating, the gene itself. Chromosomes are inherited as units, and genes on the same chromosomes usually are inherited together; this is called linkage. Two genes, however, can be separated by recombination during meiosis. The closer those genes are to each other—that is, the more closely they are linked—the less likely it is that they will be separated by recombination (see Figure 4).

The analysis of pedigrees allows geneticists to trace genes as they move through families and,

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on occasion, to infer the location of a given gene on a specific chromosome by the association of a chromosomal marker with the presence of the trait in question. Linkage analysis requires pedigrees of informative families, that is, families that have multiple affected individuals and in which certain family members are heterozygous for markers linked to the gene in question. Without these heterozygous family members, it is not possible to distinguish the chromosome that carries the disease-causing allele from its homologous chromosome. Linkage analysis established that the approximate location for the HD gene is at the tip of the short arm of chromosome 4. This gene usually is inherited along with a DNA marker called G8. In 1992, researchers still had not isolated the gene itself, even though the linkage to G8 was discovered in 1983.

MAPPING TO REGIONS OF THE CHROMOSOME

In addition to studying chromosomal rearrangements to identify the location of a particular gene on a chromosome, researchers use several other techniques.

Southern analysis. Restriction enzymes cut DNA each time they encounter a specific short sequence, usually 4 to 8 base pairs. On the average, restriction enzymes generate fragments of the following sizes: 4-base recognition sites yield pieces 256 bases long; 6-base recognition sites yield pieces 4,000 bases long; and 8-base recognition sites yield pieces 64,000 bases long. Thus, DNA treated with a specific restriction enzyme yields a collection of differently sized fragments that can be separated according to size by gel electrophoresis.

Researchers then carefully transfer, or blot, the fragments from the gel on to a nylon membrane and immobilize the fragments in single-stranded form on the membrane. Next, they expose the fragments on the membrane to radioactive probes that hybridize to any complementary DNA sequence. The radioactive probes are visible on X-ray film (see Figure 5). This technique permits researchers to identify a particular DNA fragment within a large collection of unrelated sequences and to determine the size of that fragment.

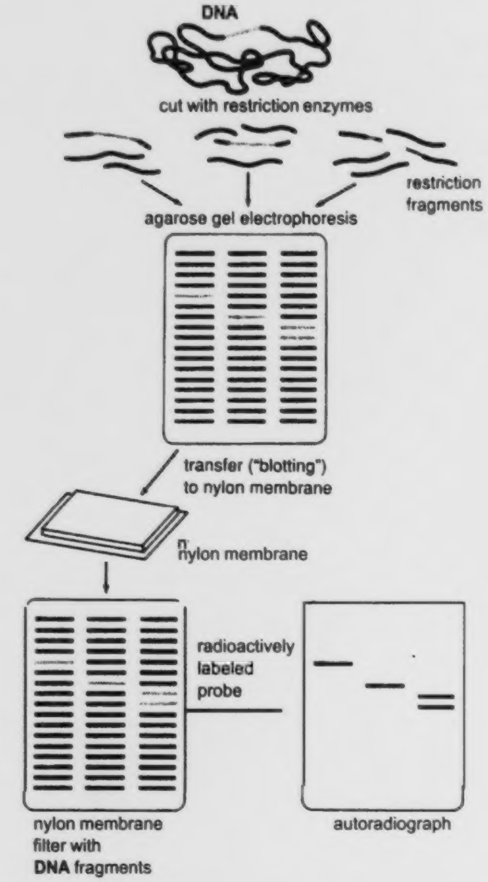
DNA
cut with restriction enzymes
restriction fragments
agarose gel electrophoresis
transfer ("blotting") to nylon membrane
nylon membrane
radioactively labeled probe
nylon membrane filter with DNA fragments
autoradiograph

Figure 5 Southern analysis allows researchers to recognize specific **DNA** fragments.

Variations in the base sequences can create or eliminate restriction sites, which will create changes in the size of the DNA blots detected by Southern analysis. These different forms of the marker—created by sequence variations—make the technique informative because researchers can detect the size differences that result from the variations. These size differences can indicate whether two DNA markers are close together or far apart on the chromosome, so one can use Southern blots to determine the relative location of DNA fragments in the genome.

Geneticists use many variations of this important technique. ("Southern" is written with a capital "S" because the technique was invented by Ed Southern in 1975. With some logic—and with tongue in cheek—related techniques using RNA or protein are called northern and western analyses, respectively.)

Yeast artificial chromosomes (YACs). This technique enables scientists to borrow the DNA

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duplication machinery of cells, including human cells. Before YACs were developed, researchers relied on *E. coli* bacteria to make millions of clones of specific pieces of DNA in which they were interested. Using bacteria, however, scientists could clone only about 50,000 nucleotides, because *E. coli* vectors cannot hold more. The development of YACs (in 1987) has allowed scientists to clone up to one million nucleotides. Only a fraction of one percent of a yeast cell's total DNA is necessary for replication. As long as these essential parts are attached to the DNA from humans or other organisms, the yeast cells is "tricked" into making copies of the DNA during its own cell division. The development of YACs makes it possible to put very large pieces of DNA into yeast cells and reproduce them in large quantities. No other cloning procedure to date can make copies of such large segments of DNA. Using YACs, geneticists need fewer clones to cover a particular region of a chromosome, and the task of determining the regions that overlap between neighboring clones is reduced greatly.

MAPPING AT THE GENE LEVEL

Direct gene detection. Identification of a gene rarely is easy. Even if researchers know the DNA sequence from a particular area of the genome that contains the gene of interest, it may not be obvious which portion of the sequence comprises the actual gene. In addition, there may be more than one gene in the same general area of the genome.

One way researchers find a gene is by using complementary DNA (cDNA), which is a DNA copy of mRNA that is made by using a viral enzyme called reverse transcriptase. Researchers can use the cDNA (see Figure 6) as a probe in a Southern analysis of DNA from the region of interest. If the probe hybridizes to the bound DNA, the gene from which the cDNA was derived is present. The reverse of this procedure is a northern analysis, where mRNA, rather than DNA, is immobilized on the membrane. The probe in this case is DNA from the region of interest. Procedures that use mRNA for direct gene detection require the use of specific tissues. Although each cell contains the entire DNA repertoire, mRNA is produced only by the genes that are expressed in that cell at that time. It would be inappropriate, for example, to search for the gene encoding a liver enzyme by using mRNA isolated from brain tissue.

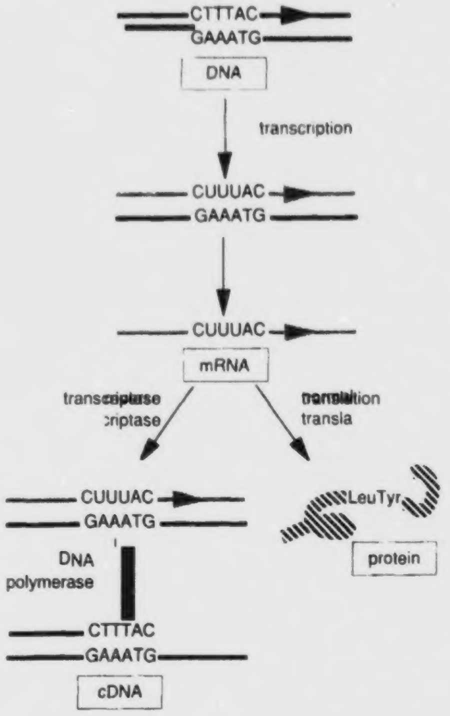

Figure 6 Synthesis of cDNA

By comparing the human genome with that of other organisms, researchers may be able to identify genes that have been conserved through evolutionary history and, therefore, may have important functions. A procedure called a zoo blot detects such genes. A zoo blot basically is a Southern analysis in which DNA from different organisms is transferred to the nylon membrane and immobilized. If a DNA probe from one organism hybridizes with DNA from other organisms, the same gene (or one very similar) may be expressed in those other organisms. Researchers isolated several human genes after hybridizing mouse DNA probes to human DNA. For example, the gene for dystrophin, the cell-membrane protein implicated in DMD, is conserved in phylogeny back to the mouse. Because zoo blots highlight conserved genes, the technique is helpful in studies of evolution.

DNA sequencing. DNA is a linear molecule composed of four different nitrogenous bases, adenine, cytosine, guanine, and thymine (abbreviated

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A, C, G, and T). The information contained in the DNA molecule is encoded in this alphabet of four bases, and researchers use sequencing techniques to determine their order in the molecule. The starting material for the sequencing procedure usually is a single-stranded DNA molecule from cloned DNA in a bacterial plasmid or from the polymerase chain reaction (see Figure 7).

DNA sequencing works because gel electrophoresis produces very high resolution separations of DNA molecules; even fragments that differ in size by only a single nucleotide can be resolved. Almost all steps in sequencing methods are now automated.

The chain termination, or Sanger method, of sequencing uses an enzymatic procedure to synthesize DNA chains of varying lengths, stopping DNA replication at one of the four bases, and then determining the resulting fragment lengths. The smallest fragment length will end up closest to the bottom of the gel. If that fragment was produced by termination of the A-chain reaction, then A is the first nucleotide in the sequence. Because smaller fragments migrate farther on the gel, the actual sequence is read from bottom to top. Figure 8 shows the Sanger method of DNA sequencing.

These first-generation gel-based sequencing technologies now are being used to sequence small regions of interest in the human genome. Although investigators could use existing technology to sequence whole chromosomes, time and cost considerations make large-scale sequencing projects of this nature impractical. The smallest human chromosome (Y) contains 50 million bases and the largest chromosome (1) has 250 million bases. The largest continuous DNA sequence obtained thus far, however, is approximately 350,000 bases, and the best available equipment can sequence only 50,000 to 100,000 bases per year at a cost of $1 to $2 per base. At that rate, an unacceptable 30,000 work-years and at least $3 billion would be required for sequencing alone.

Sequence scanning. Another way researchers find a gene simply is to scan or read the DNA sequence and look for features of a gene. This is not as straightforward as it appears, however, because the coding sequence in genes is not continuous, but appears as exons interrupted by noncoding introns. Sequences that are important for the correct synthesis and processing of mRNA may appear in the introns or in the regions on either side of the group of exons.

Although geneticists still have much to learn about the sequence features of genes, they do

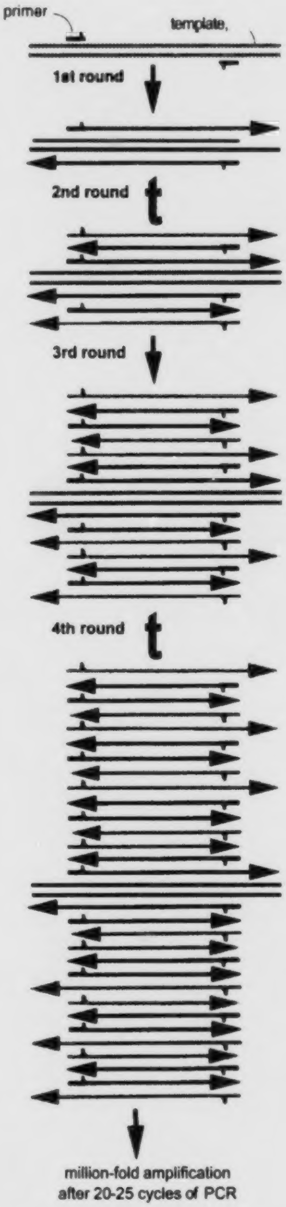

Figure 7 The polymerase chain reaction (PCR) starts with double-stranded DNA and nucleotide primers that are complementary to sequences at the ends of the fragment to be duplicated. Because of the orientation of the primers, DNA products of previous rounds act as templates in subsequent rounds. At each round the amount of DNA is doubled.

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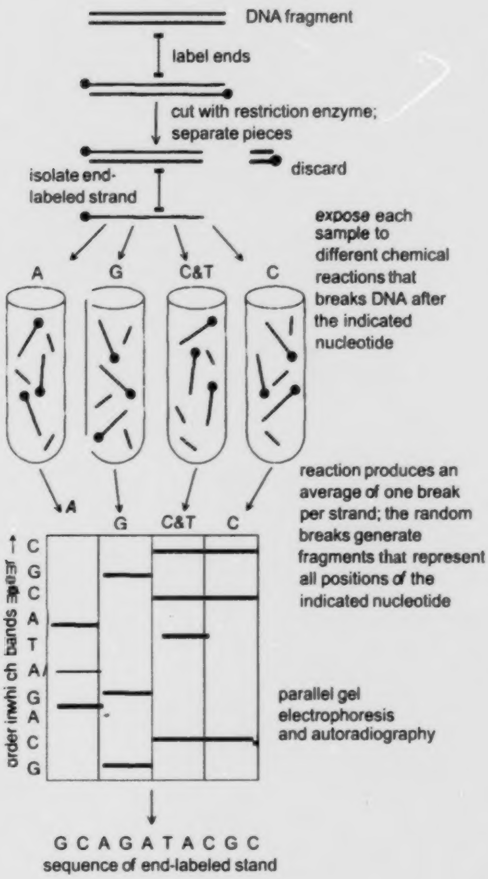
DNA fragment
label ends
cut with restriction enzyme; separate pieces
discard
isolate end-labeled strand
expose each sample to different chemical reactions that breaks DNA after the indicated nucleotide
A
G
C&T
C
reaction produces an average of one break per strand; the random breaks generate fragments that represent all positions of the indicated nucleotide
order in which bands appear
C G C A T A G A C G
parallel gel electrophoresis and autoradiography
G C A G A T A C G C
sequence of end-labeled stand

Figure 8 DNA sequencing using the chain-termination method. A different chemical reaction in each of the four samples terminates the DNA fragment (radioactive at one end) only at **A**, G, both C and T, and C respectively. The labeled DNA subfragments created by these reactions all have the radioactive label at one end and the cleavage point at the other. Electrophoresis of each sample then separates each DNA subfragment according to its size. After autoradiography of the gel, the four sets of labeled subfragments (one set in each lane) together yield one radioactive band for each nucleotide in the original DNA fragment.

know enough to do some scanning with the help of computers. For example, computers can scan DNA sequences for the presence of open reading frames—sequences that are bracketed by an in-frame triplet that codes for an amino acid and a "stop" triplet, which would signal the end of a gene. Scanning is difficult because some exons in a gene may be more than 100,000 base pairs apart, and whole genes may be contained within an intron of a larger gene.

Geneticists also look for DNA Sequences that show similarity to sequences in other organisms. These sequences, which have been conserved through common evolutionary histories, can provide insights into the function of the sequence in question. The use of sequence similarities may be misleading, however, because the functions of similar sequences may be different in different species. As the HGP progresses and our knowledge of gene structure improves, the ability to recognize genes within larger portions of the genome also should improve.

When geneticists search in a particular area of the genome for a gene associated with a genetic disorder, the appropriate gene is not always obvious. In fact, during the recent search for the CF gene, one candidate gene turned out to be a false alarm. Confirmation that a candidate gene actually is the gene of interest requires researchers to demonstrate that a mutation is present in the candidate gene *only* when the disorder is present and that the mutation *never* is present in the absence of the disorder. This is important because the human genome includes many sequence variations that do not result in disease, and researchers must distinguish between this normal variation and a disease-causing mutation.

Once researchers identify a candidate gene for a particular function or disorder, other types of experiments can help confirm its role. In such experiments, researchers determine the type(s) of tissue in which the gene is expressed by searching for mRNA—an indication of gene activity—or for proteins that would be encoded by the sequences. For example, a candidate gene for a neurological disorder generally would be expressed in the brain. Other experiments confirm the biological function of the gene. Thus, a protein expressed by a candidate gene should have a function consistent with the features of the genetic disorder.

New techniques in molecular biology will uncover many candidate genes for a variety of functions and disorders. Previously, geneticists were concerned with "diseases in search of genes," in the words of Johns Hopkins geneticist Neil A. Holtzman. Now, however, because of the growing ability to identify DNA sequences that have the characteristics of a gene, we are confronted with "genes in search of diseases."

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RESEARCH TO DETERMINE GENE FUNCTION

Once researchers identify a gene, how do they determine what it does? In some cases, scientists look for a gene in connection with a particular disorder, and thus they already know something about the gene's function. As the HGP accelerates, however, scientists will identify more genes that do not have known functions. To determine gene function, researchers can deliberately "knock out" a gene, suppress the function of a gene's protein product, or inhibit its mRNA by using complementary RNA. They then observe what happens on a cellular level, or to the whole organism. Although this clearly is not an option in humans, it has been done in other animals and provides valuable information about the function of some genes. In one study, researchers caused a deliberate mutation in a gene for a type of collagen needed for normal development in a nematode. The nematode with the mutation grew to only two-thirds of its normal body length.

Another way researchers determine the function of a gene is to compare its sequence with that of other genes and to look for similarities. If proteins or portions of proteins have similar structures, they may have similar functions. Examination of the protein structure itself also may provide some clues. For example, a stretch of hydrophobic amino acids may indicate a protein that is attached to the lipid cell membrane.

Examining the tissues or cells in which a gene is expressed by looking for mRNA, or examining the stage of development at which the gene is expressed, also may provide some clues about the gene's function. In addition, geneticists may examine the protein product itself to determine its biochemical properties. Without other indications of gene function, however, such experiments are trial-and-error.

HUMAN GENETIC VARIATION

Intron variation. The goal of the HGP is not to provide the sequence data from a single individual, but rather to provide a collection of sequences from many different people. As shown in Table 2, there are many differences in the genome from one person to the next. Indeed, except for identical (monozygotic) twins, no two people have identical genomes. Most sequence differences occur outside the coding regions (in the introns) and have no detectable effect on the phenotypes. In fact, molecular biologists estimate that only five percent of the genome contains DNA sequences that code for protein. Sequence variation can be beneficial, neutral, or harmful. Beneficial variations, of course, provide the foundation for evolution by natural selection.

Table 2 The Extent of Human Genetic Variation

Any two people differ at about 0.1 percent of their DNA bases. Thus, 0.001 (0.1 percent) x 3 x 10^9 base pairs = 3 x 10^6 base pairs of variation between any two individuals.

There are approximately 5 billion (5 x 10^9) people on earth. Thus, 3 x 10^6 x 5 x 10^9 = 15 x 10^{15} base pairs of potential variation in the human population.

Exon variation. Other types of sequence variations occur within the coding region of genes, but have little or no effect on the final protein product. Because different DNA triplets can code for the same amino acid, a base variation may not result in a change in the protein. Sequence variation also may change one amino acid to a very similar one without altering significantly the function of the protein (a "conservative" change). In addition, an amino acid change may occur in a region of the protein that is not important for function, as Activity 1 illustrates.

Protein variation. A sequence alteration or mutation can change a protein significantly if the change occurs in an important area. Such mutations often result in disease or abnormality, and a mutation can disrupt a gene in any number of ways. A change in the amino acid sequence can alter or eliminate the function of the protein (a missense mutation), or one of the DNA triplets may be changed to a stop triplet, which results in a truncated protein product (a nonsense mutation). Alterations also may occur in one of the regions outside the coding sequence that is important for the expression of the gene, for example in the promoter, where RNA polymerase initiates the transcription of the gene. As a result, no protein is produced at all. Also, the process of gene expression from DNA through RNA to protein may be disrupted in some way, such as the incorrect removal of the introns. There even may be some major DNA alteration, such as a large deletion that removes the gene completely. As Activity 1 demonstrates, the same disorder can result from different mutations in the same gene.

Population variation. Some sequence variations are associated with specific populations. Research in population variation at the molecular

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level affects theories of human evolution because it is possible to develop and test hypotheses about the derivation of one population from another.

Certain genetic disorders occur more frequently in some populations than in others, and this knowledge affects the development of cost-effective screening programs for specific high-risk groups, when the disorder is severe and the screening can provide some concrete benefit. For example, Tay-Sachs disease occurs with a much higher frequency in Ashkenazi Jews (Jews from Eastern Europe) than in the general population. Similarly, sickle cell disease occurs most frequently among Blacks and among Hispanics of Caribbean ancestry. Medical geneticists often screen members of these particular populations for carriers of these serious disorders.

Although DNA sequence variation often is recognized because it results in a disorder that requires medical management, there are some biological advantages to genetic variation. Evolution, for example, depends on genetic variation in the members of a population in each generation. This variation occurs at the molecular level because of errors in DNA replication and through genetic recombination during sexual reproduction. The majority of the variations produced by replication errors have no effect on the phenotype, but some variations are disruptive and result in disorders. Other variations result in harmless differences between individuals and thus create the possibility of an advantageous trait that may survive natural selection.

In addition to the biological advantages, variation is useful in fields such as forensic science. In 1985, for example, British molecular biologist Alec Jefferies developed DNA fingerprinting, which exploits extensive variation in some regions of the genome. It is possible to take a tiny amount of tissue, for example, a hair root left at the scene of a crime, and use PCR in combination with Southern analysis to create the DNA profile of the person from whom the sample came. If a suspect is identified, scientists can obtain a DNA profile from a sample of blood and use it to demonstrate the suspect's innocence if the profiles are different or provide strong evidence for conviction if the profiles are the same. Although DNA-based evidence has gained wide recognition in the courts, it still is considered controversial because of the lack of standards in its application and interpretation. An April 1992 report from the National Academy of Sciences proposed new standards that should bring more consistency to the use of DNA fingerprinting in criminal cases.

The United States military also has used DNA fingerprinting as a DNA "dogtag." DNA profiles of individuals in the military make possible the identification of fragmentary remains. This technique was used to identify some of the individuals who died in the Persian Gulf War.

EXPECTED RESULTS FROM THE HUMAN GENOME PROJECT

Biologists already have some ideas about possible results of the HGP, but there probably will be some surprises as well.

Diagnosis and prediction of disorders. Geneticists isolated (cloned) genes responsible for genetic disorders even before the organization of the HGP. Recent, well-publicized successes include the cloning of genes responsible for DMD, retinoblastoma, CF, and neurofibromatosis. If other such disease-related genes are isolated, biologists can learn about the structure of the gene's corresponding protein and the pathology of the disorder. This knowledge could lead to better medical management of the resulting disorders.

Several techniques to detect gene mutations allow immediate and accurate diagnosis in individuals with some symptoms. For example, before geneticists cloned the DMD gene, the confirmation of a diagnosis required expensive and uncomfortable tests, and the tests were inadequate to detect carriers. Now, with only a blood sample, geneticists can detect most mutations associated with DMD very rapidly. DNA-based tests clarify the diagnosis quickly and enable geneticists to detect carriers within the same family.

The probability of erroneous results from a genetic test is small, but not zero; false-positive or false-negative results can occur because of technical abnormalities or human error such as the mislabeling of samples. In addition, some tests such as that for CF cannot detect all of the mutations associated with the disorder. The use of genetic tests to detect carriers, for prenatal diagnosis and for presymptomatic diagnosis, has created difficult ethical and public policy issues, some of which are discussed in Section II.

Genome information also can indicate the **future** likelihood of disease. For example, if the gene responsible for HD is present, it is a near certainty that symptoms eventually will occur, although geneticists cannot predict accurately the time of onset. Genome information also helps

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geneticists predict which individuals have an increased susceptibility to disorders such as heart disease, cancer, or diabetes, that result from complex interactions between genes and the environment. There is no guarantee that symptoms will occur, but the risk is greater for individuals with specific genotypes than for the general population.

Insights into basic biology. Information generated by the HGP may shed light on several interesting biological questions. The discovery of new genes is an obvious benefit, as is the determination of their functions. The organization of genes within the genome is another area of investigation in biology. Is it important, for example, for genes to reside on a particular chromosome, or in a particular order, or both?

When biologists compare the human genome with the genomes of other organisms, they may gain some insight into molecular evolution, including human evolution. Comparisons of human and mouse DNA sequences will help identify genes that are unique to one or more complex organisms, and comparisons of DNA sequences from humans and fruit flies or nematodes may help identify genes essential for all multicellular organisms. Comparisons of human and yeast DNA sequences may help identify genes related to functions essential for all eukaryotic cells.

Development of new technologies. The HGP has catalyzed enormous advances in the development of technology, and it will continue to do so. These advances already have had considerable impact outside of the HGP.

The polymerase chain reaction, or PCR, was invented in 1985 by biochemist Kary Mullis. This technique multiplies a particular region of DNA and generates a large quantity of DNA from a small tissue sample. A DNA *polymerase* synthesizes the DNA, and the products of one reaction become the starting material for another, resulting in a *chain reaction—a* repeating reaction that feeds itself (see Figure 7 on page **8**).

DNA has the ability—in the presence of appropriate intracellular enzymes—to direct the synthesis of a complementary strand from one strand of the original double helix (the means by which DNA normally is replicated in the cell). A DNA strand cannot start from scratch, however, so a short nucleotide sequence, called a primer, must be added to the end of an already existing chain. Once the primer is in place a, DNA polymerase catalyzes the synthesis of a new DNA strand.

In PCR, one primer is complementary to the sequence at one end of the DNA region under study, and the other primer corresponds to the complementary strand of DNA at the opposite end. In the PCR itself, one primer directs the synthesis of one strand of DNA complementary to the original DNA. The second primer directs the synthesis of the opposite strand. Additional rounds of synthesis copy the DNA over and over, using the products of the first round of synthesis as a model and new primers.

One of the biggest challenges is finding faster ways to sequence DNA. One as yet unproven idea uses a DNA "chip." The chip is not a piece of electronic equipment, but an array of short pieces of DNA, each with a known sequence, arranged on a substrate. When a solution containing DNA with an unknown sequence is applied to the substrate, some of it reacts with the short DNA whose sequence is known. The signal indicates to which unknown sequence the known sequence hybridized. This yields a pattern that a computer can use to determine the sequence of bases.

By one estimate, the HGP ultimately will provide 10 million times more data about each individual than is available at the chromosome level, and researchers are developing computer hardware and software necessary to manipulate the huge quantity of data. One important addition is the Genome Data Base, located in Baltimore, which incorporates data from human genetic maps as they accumulate. GenBank®, in the U.S., and several other data bases around the world accumulate sequence data from a variety of organisms.

Researchers have made great progress in developing automated machines and robotic work stations to perform repetitive procedures. These machines are particularly useful in large-scale sequencing operations, and computers can read the results of a sequencing experiment and load the data directly into a data base. Although automation accelerates the procedure and reduces the opportunity for human error, human involvement still is necessary.

LIMITS AND OPPORTUNITIES

Determination of the entire DNA sequence contained in the human genome will not answer the question: What is a human? Geneticists will not be able to look at a person's DNA sequence and predict everything about the appearance and characteristics of that person. Even if geneticists can identify segments of DNA as genes, the vast majority of the genes they discover still will have

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unknown functions. In addition, many human traits such as body stature and intelligence result from multiple genes, and the exact number of genes that might contribute to such a trait is not obvious, nor are the ways in which those genes interact. An individual's genetic make-up greatly contributes to the type of person he or she is, but environmental variables such as diet, education, climate, family values, and access to health care also play a considerable role in determining an individual's characteristics.

As of 1992, geneticists had identified more than 5,000 single-gene traits in humans, including disorders such as CF and DMD. Even in single-gene disorders, however, there usually is considerable variation in the expression of the gene. This variability may result from different mutations in the same gene, environmental effects, interactions with other genetic features, or any combination of these factors. Thus, even when geneticists discover a disease-related gene in an individual, they cannot always predict the exact course of the disorder.

Although it may appear that our genes are relatively stable, the human genome changes continually because of errors in DNA replication. Genes responsible for genetic disorders may be inherited from one or both parents, or they may arise from new mutations because of errors in the replication of an individual's DNA. It is unlikely, therefore, that a geneticist could absolutely exclude the possibility of a genetic disorder by examining an individual's genome. New mutations are more likely in X-linked and autosomal dominant disorders than in recessive disorders.

There are certain areas of human genetics in which researchers expect to expand their current knowledge. Two such areas are the regulation of gene expression and the role of the vast majority of DNA that has not yet been assigned a function—the inappropriately named "junk" DNA. Research published early in 1992 demonstrated that an intron plays a role in the function of transfer RNA, which is critical to protein synthesis. Additional research likely will reveal other functions for such "junk" DNA.

Geneticists already have made one surprising observation from their increased knowledge of the human genome—a phenomenon called imprinting. Imprinting describes a molecular signal that indicates whether one allele of a pair was inherited from the mother or the father. Apparently, in some cases it is not sufficient merely to have two copies of a gene. Rather, each copy must be inherited from a different parent. Imprinting is implicated, for example, in Prader-Willi syndrome and Angelman syndrome. Individuals with either of these disorders are mentally impaired. Infants with Prader-Willi syndrome usually are small at birth and may experience respiratory and feeding problems. They become obese as young children, their skin is sensitive to light, and their hands and feet are small. Children with Angelman syndrome have an abnormal puppetlike gait and sudden outbursts of inappropriate laughter. These two dissimilar disorders apparently result from a disruption of the same region on chromosome 15, but in Prader-Willi syndrome, the mutation is inherited from the father, whereas in Angelman syndrome, the mutation is from the maternal side.

In addition to expanding our knowledge of genetics, the HGP also will provide a number of new job opportunities in biotechnology, health care, computing and information storage, and ethics and public policy. Discussions of ethics and public policy will require input from individuals trained in disciplines such as philosophy, theology, law, medicine, science, sociology, and public policy.

CONCERNS ABOUT THE HUMAN GENOME PROJECT

Critics express several concerns about the HGP and most involve the content of the project itself, or its funding. Original proposals for the project emphasized sequencing the entire human genome. This goal, however, is controversial because of the high cost and because many critics believe that sequencing a huge amount of noncoding DNA should have low priority in a time of limited funds for research. On the other hand, most individuals involved in the project agree that detailed genetic and physical maps would be extremely useful. Therefore, mapping of the genome now is the primary goal, with complete sequencing to follow only if the cost becomes reasonable.

Only about 5 percent of the genome contains sequences that are coding regions, and some biologists still maintain there is little point in sequencing the other 95 percent. Because biologists already know that several regulatory signals are in noncoding regions of DNA, a compromise has been reached. A few pilot sequencing projects are focusing on sequencing certain coding regions that are most likely to contain information valuable to the medical and biological communities.

A major criticism of the HGP is similar to that raised against other mega-science projects such as the space station or the superconducting

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supercollider: the high cost is not justified. This big science vs. little science argument maintains that funding such large-scale projects takes scarce resources from researchers who may study certain areas of particular interest more efficiently. Conversely, others argue that coordination of the HGP is a more efficient way to conduct research in human genetics because it minimizes duplication of effort. Still, at this point, the HGP funds research only on mapping and sequencing, not on gene function.

Some critics suggest that the ability to diagnose a genetic disorder before any treatment is available does more harm than good because it creates anxiety and frustration. Indeed, geneticists have isolated several disease-causing gene mutations and have studied them in great detail without developing a treatment. For example, the mutation in the beta-globin gene that results in sickle cell disease was identified in 1956, but there is no treatment as yet. Scientists eventually may develop successful therapies, but until they do, this criticism is significant. Even in the absence of new treatments, however, the HGP may make diagnosis possible before the onset of symptoms and, thus, make management of the disorder more effective. In addition, improved preconceptual analysis of the parents' genotypes can provide couples with a broader range of options for family planning.

Some critics of the HGP maintain that social and political mechanisms to regulate the ultimate outcomes are insufficient. Because of the genetic variation between individuals, there never will be one definitive human sequence. The lack of a definitive sequence creates uncertainty about the appropriate definition of "normal," which, in turn, makes the discussion of public policy issues difficult. Questions about controlling the manipulation of human genetic materials concerns these critics, as does the idea that simply because these scientists *can* do this science, they *ought* to. These critics point to the development of atomic weapons and argue that the science that led to their development caused far more problems than it resolved.

Few religious groups in the United States formally have addressed the specific ethical and public policy issues raised by the HGP, although there is active interdenominational discussion of issues related to human genetics in general. Public policy debates are enriched considerably by input from these various groups.

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Section 11
Ethical and Public Policy Dimensions of the Human Genome Project

The uses of information from the HGP will be numerous, and they will affect us in a variety of ways, only some of which we can predict reliably at present. Individuals, institutions (schools, businesses, and other organizations), and society will have to deal with situations in which some interests are advanced and others are impaired. When the interests of everyone cannot be advanced, and when some interests are advanced at the expense of others, whose interests ought to receive priority? Questions about "oughts" properly are addressed by ethics and public policy. Because such questions will continue to arise from the HGP, it is essential to understand the ethical and public policy dimensions of the HGP to understand the project itself.

The HGP has pledged at least three percent of its annual budget to support research, discussion, and proposals about the societal implications of findings from the HGP. A working group composed of members from the Department of Energy, the National Institutes of Health, and other organizations is coordinating these efforts. This group is called ELSI—the acronym for Ethical, Legal, and Social Issues related to mapping and sequencing the human genome.

This section addresses a number of important, highly probable uses of information generated by the HGP, and for each use, it identifies some major ethical and public policy questions. (Students will do this for themselves as part of Activity 3 and Activity 4.) These questions set the stage for a more detailed consideration of ethics and public policy and the most effective way to teach them in the classroom.

USES OF DATA FROM THE HUMAN GENOME PROJECT: NEW QUESTIONS FOR INDIVIDUALS AND SOCIETY

Issues Related to Organization and Access. Geneticists will use genome data collected from many people to define the general human genome (aggregate genome data) and genome data collected from one individual to define a personal genome. Whereas the general availability of the former accelerates research and prevents duplication of effort, the latter is, in effect, a medical record that raises various issues, including confidentiality.

Personal genome data. An individual's personal genome data are unlikely to consist of a complete base sequence (for many years at any rate). The data probably will consist of portions of sequences from key regions of the genome—most likely genes that affect susceptibility to disease. Activity 2 illustrates some of the ways in which a partial genetic DNA profile might be used at some future time. Activities 3 and 4 illustrate the real-life implications of organizations and agencies having access to personal genome data. Questions about the uses of personal genome data include the following:

- Should employers be permitted to discriminate against job applicants on the basis of a genetic predisposition for later onset of a disabling disorder? (This is the central topic for discussion in Activity 3.)
- Should private insurance companies be permitted to issue or deny policies based on such information?
- Who should have access to personal genome data?
- What concerns arise about informed consent for obtaining personal genome data as a result of the incomplete and changing knowledge of the human genome?

Aggregate genome data. The task of organizing data from the HGP is daunting. Data bases containing human genetic information already exist, including a catalogue of genes, details of the human genetic map, and two lists of DNA and protein sequences. As the data bases currently are organized, they are inadequate to manage the huge influx of data expected, but technologists are developing systems to cope with more information. The Human Genome Program Centers are focal points for the accumulation of data and materials. Questions that relate to the uses of aggregate genome data include the following:

- How can we ensure that information in very large data bases is used appropriately when many individuals have access to the information?
- Should state or federal governments have the responsibility to prevent labeling of or unfair discrimination against individuals or groups based on genetic information?

MEDICAL AND ECONOMIC ISSUES

Diagnosis of disorders. Genome information can clarify diagnosis of genetic disorders such as DMD or CF (an example used in Activity 1).In any case, a correct diagnosis is important for appropriate medical treatment and it is essential in counseling family members about their risks for the same disorder. Questions that relate to diagnosis include the following:

- Is it beneficial to patients to diagnose a disease for which there is at present no treatment, as is the case, for example, for HD?
- Once geneticists discover the genes for a large number of genetic disorders, which disorders should receive priority for research efforts aimed at finding effective treatments?

Prediction and prognosis of disorders. Personal genome information can predict the occurrence of a disorder such as HD, indicate an increased susceptibility to a disorder such as heart disease, or indicate the severity of a genetic disorder, as in DMD. Among the questions related to prediction and prognosis are the following:

- Should researchers or funding agencies give priority to research in the *treatment* of genetic disorders, or to research in the *prediction of the severity* of genetic disorders?
- Who should decide whether to use a particular predictive test in a specific individual?

Genetic testing and screening. There is an important distinction between genetic testing and genetic screening. In the former, geneticists test the relatives of an affected individual (or a fetus) for that same disorder to determine their carrier status or risk of becoming affected themselves. In population-wide screening, geneticists test individuals in the population for the disorder in question regardless of family history. If population-wide screening occurs in the absence of adequate education or counseling for individuals and society as a whole, there is a great possibility of misunderstanding or unjustifiable discrimination. Questions about genetic testing and screening include the following:

- Do individuals who have a family history of a serious genetic disorder have an obligation to future children and to society to undergo genetic testing?
- Should genetic information such as carrier status or paternity information always be provided to family members who did not seek the information for themselves?
- When family testing is necessary for the diagnosis of a genetic disorder in an individual, is it permissible for the courts to order reluctant family members to undergo testing?
- How accurate should screening procedures be before they are made available for widespread use?
- Should genetic screening ever be mandatory?

Gene therapy. Gene therapy is the insertion of a functional gene into a cell that lacks that function. Through germ-line therapy, which involves egg and sperm cells, scientists can alter the entire genetic makeup of an organism so the individual passes the genetic change to his or her offspring. This procedure is controversial in humans and at present is not approved for clinical trials. Somatic cell therapy, which involves cells other than eggs and sperm, already is undergoing clinical trials because this therapy repairs only the affected tissue(s) and is somewhat analogous to an organ transplant. One of the potential risks of both types of gene therapy is the possibility of unknown genetic effects, such as cancer.

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One of the first disorders medical specialists treated using somatic-cell gene therapy is a deficiency of the enzyme adenosine deaminase (ADA), which results in severe combined immunodeficiency disorder (SCID), the disorder that affected David, the "bubble boy." Bone-marrow transplants often can cure this disorder, but few patients have a closely matched donor, and thus the risk of rejection and other complications is increased. In gene therapy for ADA deficiency, surgeons remove bone-marrow cells from the patient. Researchers incorporate the missing gene into their genome (using a safe virus as the vector) and the surgeons then return these cells to the patient. Because they now have a functional ADA gene, these cells outgrow the original ADA-deficient cells and repopulate the bone marrow with immune-competent cells. Questions that relate to gene therapy include the following:

- When is it justified to use somatic-cell gene therapy given that the therapy carries the possibility of unpredictable risks?
- Would it be justified to perform germ-line therapy to eliminate a lethal single-gene disorder when the therapy itself carries unpredictable risks for all descendants?
- What role should the government play in regulating somatic-cell and germ-line therapy?

Reproductive decision making. Knowing whether you or your mate is a carrier for a genetic disorder can affect family-planning decisions. Because the HGP will permit prenatal detection of many more genetic disorders, individuals may choose to have a genetic test performed on a fetus early in pregnancy. It also may be possible that individuals who intend to marry, or couples who are married, will choose to be tested to identify their carrier status. The HGP will provide far more information than presently is available and will raise questions such as the following:

- Do couples who have a family history of a genetic disorder have an obligation to undergo genetic testing before conceiving offspring?
- When the HGP allows us to identify a large number of genetic characteristics, should the government develop policies to regulate the dissemination of the information?
- How will new information from the HGP influence the ethical and public policy debates about abortion?

Pharmaceutical and biological products. Information and materials from the study of the human genome already have produced new and better pharmaceutical products and, as the HGP proceeds, more products are likely to appear. An important advance for the pharmaceutical industry is the isolation of genes for proteins such as blood products or growth hormone that are absent in various deficiency disorders. In the past, these proteins were isolated from human blood or cadavers and had the associated risks of viral infection such as AIDS or hepatitis. The availability of recombinant protein products from cloned genes has eliminated these risks. Recombinant protein products also could reduce or eliminate the need for the isolation of products from fetal tissue—a very controversial issue.

Once researchers isolate a disease-related gene and determine the structure of the corresponding protein determined, researchers may be able to design better drugs for treating that disorder. For example, scientists already have developed a drug called alpha interferon to treat hairy-cell leukemia. Questions that relate to the development of biological products include the following:

- If generating recombinant proteins does not involve ethical and public policy controversies such as those surrounding the use of fetal tissues, is this a reason to give these techniques higher priority for research and development?
- What role should the government play in regulating research on recombinant protein products, especially when it involves the use of human research subjects?

Profit. Individuals and several types of organizations, particularly those that develop new technologies or pharmaceutical and biological products, may profit from the HGP. The massive investment of public monies in the genome project makes these profits possible. The potential for profits from the HGP raises questions such as the following:

- Should scientists withhold the results of their work rather than share results with the rest of the scientific community because of potential profit?
- To what extent should the government regulate scientific research in private industry when the government has funded the basic research?
- Should taxpayers have to pay twice for the benefits of the HGP—once to support federally funded research and again to purchase the products or services based on that research?
- To what extent should public policy recognize intellectual property rights, such as patents, copyrights, and trade secrets of researchers and companies?

Education. The HGP will pose significant educational challenges. Individuals will be dealing

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with complicated scientific, technological, and clinical questions as part of the informed-consent process for the mapping and sequencing of their personal genomes. Health-care professionals, who heretofore have concerned themselves with genetics only tangentially, may be required to provide a genetic context for the diseases that affect their patients. Society as a whole will face the difficult task of educating itself about genetics and about the promises and pitfalls of the HGP. Other education-related questions include:

- Should all health-care professionals be required to learn about genetics and its role in morbidity and mortality?
- Could the sheer amount of information that individuals must understand undermine their ability to provide valid consent to genetic testing or screening?
- What resources should society commit to the education of children and adults about the HGP and to continuing education for all about the unexpected results of the HGP?

Conceptual issues. The science and technology of the HGP raise issues of importance about the philosophy of science and technology. Some of the information that will be available as a result of the HGP may require that society reevaluate and perhaps modify some of its fundamental concepts regarding individual rights and responsibilities. For example:

- Given the extent of human genetic variation and the large number of people who will be discovered to have disease-related genes, how will we come to view the concept of genetic disease? What view will be most appropriate for protecting individual rights?
- Given new insights into the interactions of genotype and environment that the genome project may illuminate, how does new information about genetic factors that influence human behavior affect the concept of individual responsibility for one's actions? In general, the HGP and the information it generates will extend debate about the power we ascribe to DNA to determine complex characteristic — the very language we use to describe the HGP and its products will affect the public's perception of genetics and human biology.

The foregoing questions concern what society should or should not do in response to the issues raised by the HGP. What society should do defines our duties. A duty or obligation is something an individual is bound to do because it is the right thing to do, and as such, duty justifiably restricts freedom. To understand the ethical and public policy dimensions of the HGP, individuals must address the following questions:

- What is ethics?
- What is public policy?
- How are ethics and public policy related to each other?

The student materials are designed to address these questions in the classroom. Each student will confront these issues directly or indirectly for the rest of his or her life, and the intellectual skills developed in this module are essential to becoming a responsible citizen in response to the ethical and public policy challenges of the HGP.

ETHICS

Ethics is the study of what is right and wrong and what is good and bad, applied to the actions and character of individuals, institutions, and society. Although ethics often is considered an esoteric, difficult discipline that has little practical value, you and your students make ethical judgements all the time. Ethical analysis simply is the conscious analysis and discussion of the justifications for our decisions.

Generally in ethics, we apply the terms *right* and *good* to those actions and qualities that foster interests; we apply the terms *wrong* and *bad* to those actions and qualities that impair the interests of individuals, groups, or society. During the last 2,500 years, Western philosophy has developed a variety of powerful methods and a reliable set of concepts and technical terms for studying ethics.

The features of ethics. Experts generally agree on the following features of ethics and the teaching of ethics. First, ethics is a process of ***rational inquiry.*** Inquiry involves posing questions. Rational inquiry in ethics, as in science, involves posing clearly formulated questions and seeking ***well-reasoned*** answers to those questions. Well-reasoned answers to ethical questions are the result of successful ethical inquiry.

Second, ethics requires a solid foundation of information. In particular, to ask and answer questions about the ethics of the HGP requires a solid understanding of genetic science and technology. Ethics, thus, is not strictly a theoretical or abstract enterprise, but also is concerned with practical matters.

Third, because trade-offs among interests are complex, constantly changing, and sometimes uncertain, there often are competing, well-reasoned

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answers to questions about what is right and wrong or good and bad regarding complex matters such as the HGP and its applications. Again, this parallels scientific inquiry, as in the ongoing debate about the relative influence of genes and environment in the development of complex traits.

Genetic variation and its variable expression in individuals is a fundamental feature of the human genome. Because this variation makes accurate predictions about genetic predisposition to many diseases very difficult, because genetic tests themselves have varying degrees of certainty, and because individuals have different value systems, the most frequent outcome of ethical inquiry about the HGP will be competing, well-reasoned answers. This makes suspect any claim to a final, irrefutable answer to ethical questions about the science and technology of the HGP.

How to talk about interests. The concepts and terminology of ethics that are relevant to the HGP concern different ways of talking about the interests of individuals, institutions, and society. There are **two** ways to talk about these interests. One way is in terms of the results or *consequences* of actions. Synonyms the students might use include *results, outcomes, effects, purposes, goals,* or *ends.* Rational discussion about consequences requires that individuals be able to give well-founded reasons to explain why society should or should not pursue the consequences of an action. Consequences that advance interests are labeled right or good, and society should pursue such consequences. An example from the HGP is the potential for increased knowledge of the genetic contributions to disease, which then would advance the public-health interests of society. Consequences that impair interests are labeled wrong or bad, and society should not pursue such consequences. An example from the HGP is the potential for an individual to discover that he or she has a fatal genetic disease for which there is no treatment at present. This would impair the interests of that individual.

Another way to talk about interests is in terms of a *right* or *rights.* A right is a claim to be treated in a certain way regardless of the consequences in doing so. Synonyms the students might use include *claim, entitlement, power, liberty,* or *freedom.* An example of a right pertinent to the HGP would be a refusal by an individual to submit to genetic testing for HD despite the consequences of not knowing his or her genetic status.

Respect for rights promotes interests because it allows individuals to pursue things that society values. Society should pursue respect for rights. Denial of rights damages interests because denial does not allow individuals to pursue what society values. Denial of any right should be prevented. At the same time, denial of some rights may be justified when rights are in conflict. Rational discussion about rights requires that individuals be able to give credible reasons to explain why society should respect or may deny the right in question.

The role of argument in ethical inquiry. Ethics seeks to establish what should or should not be done on the basis of rational argument. An argument is a set of clearly stated premises or reasons that together *justify* a conclusion. All credible arguments must meet two standards.

The first standard is *validity.* An argument meets this standard when the conclusion follows from the reasons by the accepted rules of logic. The classroom activities for this module are designed so that validity is satisfied in all cases. The principal advantage of the design in this module is that you do not have to teach the formal rules of logical inference to your students.

The second standard requires that *all premises or reasons Count as good reasons for everyone.* To satisfy this standard, a student must be able to say why everyone should accept that reason as important and relevant to the issue at hand. It is not enough for a student to say that the reason is important to him or her. Just as scientific evidence must be made public, the reasons for ethical arguments also must be made public. The classroom activities in this module place a great emphasis on meeting this standard.

Reasons that are supported by religious arguments deserve special consideration. Students always should respect their classmates' religious beliefs and convictions. The moral content of religious beliefs and convictions frequently is expressed in terms and concepts that are important only to the person making the argument or to one's particular faith. It is an essential teaching device, however, to have students try to express a particular religious belief or conviction in terms that are important to everyone. If this process succeeds, the reason counts as important to all. If this process does not succeed, the reason does not count as important to all and cannot be used in an argument. This result *in no way* affects its importance for the student who offered it or the obligation of the other students to acknowledge and respect that importance. Denigration of any religious belief is inconsistent with respect for stu-

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dents and should not be tolerated in the classroom. Teachers should take this same approach with all expressions of serious moral convictions, including those of non-religious origins.

PUBLIC POLICY

Public policy is a set of guidelines or rules that results from the actions or lack of actions of governmental entities. Governmental entities act by making laws. Laws can be made by legislatures (statutory law), by courts (common law), and by regulatory agencies (regulatory law) at the local, state, and federal levels. All three types of law are pertinent to the HGP. The law will be concerned for the most part with the regulation and funding of the HGP and its applications. When public policy is a function of law, it is called *de jure* (according to law) public policy. *De jure* public policy may be subject to ongoing ethical inquiry.

Governmental entities also can make public policy by *not* acting. When governmental entities deliberately or by accident do not act, the effect on public policy is to permit individuals and institutions to act in the ways they choose, without the interference of law. Given the complexity and the very large scale of the HGP, it is unlikely that many aspects of the project will be regulated explicitly by laws. When public policy is a function of the lack of action by governmental entities, it is called *de facto* (actual) public policy. The *de facto* public policy aspects of the HGP will be subject to ongoing ethical inquiry.

TEACHING ETHICS AND PUBLIC POLICY IN THE CLASSROOM

Ethics is vital to *de facto* public policy because it provides the concepts and terminology for the carefully organized debate that can result in well-reasoned conclusions about what society should or should not do. This inquiry is valuable in and of itself. Once society identifies a well-reasoned conclusion, it is reasonable to ask whether it should be enacted into *de jure* public policy. Sometimes the best public policy response is *not* to enact a law in response to a controversy, but rather to allow individuals, institutions, and society to act in the manner they choose.

The teaching materials for this module are organized according to five convenient steps for teaching ethics and public policy in the classroom. Steps *1-4* involve teaching ethics as a process of rational inquiry; step *5* involves teaching the students how they can translate the conclusions of ethics into public policy.

- Step *1—information gathering*. Science is the key to this step because genetics and technology provide ethics with the information about the HGP and its applications.
- Step *2—evaluation of the information gathered in step 1*. This step involves the evaluation of information from the HGP and its application in terms of the impact of the HGP on the interests of individuals, institutions, and society. Activities *3* and *4* involve this step. In Activity *3*, we ask students to think in terms of both rights and consequences as they make arguments in response to the questions the IPC Employment Selection Committee pose to them. In Activity *4*, the students must think about rights and consequences in relation to a proposed law that would preclude the sale of alcohol to certain individuals based on their personal genome.
- Step *3—making ethical arguments*. An argument is a set of clearly stated reasons that together justify a conclusion. An argument begins with reasons and ends with conclusions. Portions of Activity 3 emphasize the need for students to justify the premises or reasons in their arguments by stating why those reasons should count as important to everyone. Activity *4* also requires that students perform this step. Expect your students to put forth a variety of reasons to meet this standard. Also expect the same conclusions to be supported by different reasons. Obviously, different reasons can lead to different conclusions.
- Step *4—analyzing arguments*. The students should compare their arguments and attempt to produce the most well-reasoned argument. Both Activity *3* and Activity 4 are designed to promote this process.
- Step *5—translating the results of ethical arguments into public policy*. Whether any conclusion of a well-reasoned ethical argument should become *de jure* public policy depends on whether that conclusion satisfies the following conditions for enacting that conclusion into law: urgency, means, and effectiveness. For the purposes of these activities, however, we ask students to consider only urgency and effectiveness. (If the public policy is seen as effective, then we assume there are means in place with which to address the situation.) If conclusions from ethical arguments do not satisfy these conditions, it is not reasonable to enact a law. Instead, ethical inquiry should continue and public

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policy should remain at the ***de facto*** level. These activities are designed to provide the students with an experience of applying these conditions to the conclusions of their own ethical arguments.

Condition 1.

The situation is urgent: There is immediate risk of serious, far-reaching, and irreversible harm if the legislation is not enacted or the law is not changed.

Immediate means that there are reasonable scientific grounds to conclude that impairment of interests will occur in the near future, for example, access by unauthorized individuals or institutions to human genome data bases will deny or violate individuals' rights of privacy.

Serious means that the risk involves potentially grave injury to interests, for example, some private insurance carriers may deny coverage to an individual who has a genetic predisposition to a particular disease.

Far-reaching means that the impact of severe impairment of interests may be widespread, for example, a public policy of mandatory genetic testing of all pregnant women or children at birth would threaten the interests of millions of individuals.

Irreversible means that the serious damage to interests likely will be permanent, for example, labeling infants as having a genetic predisposition to learning disabilities could well have permanent impact on their future education.

Condition 2.

There are effective means to address the urgency of the situation: Scientifically valid or technologically practical means are available to prevent, reduce, or avoid risk of serious, far-reaching, and irreversible harm. The public policy must work and be enforceable—resources must be available to implement the public policy. An example would be that there are enough scientifically and technologically qualified individuals to carry out population-wide genetic screening. Public policy works when it enjoys broad-based political acceptance, that is, when few, if any, individuals or groups disagree strongly with the public policy. An example of a policy that is controversial in this respect is requiring all job applicants to submit their individual genetic profile to prospective employers. Public policy is enforceable when few, if any, individuals or groups will disobey the public policy. An example of policy that is controversial in this respect is a requirement that all citizens submit their individual genetic profile to the federal government for inclusion in a national data base.

It is our intent that the following activities will help provide your students with the knowledge and skills required to understand the HGP at a basic level and to analyze the relevant issues in a manner that fosters informed, respectful debate and sound decision making.

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Glossary

This list of abbreviations and definitions is intended to help you understand the teacher's narrative; it should *not* be used as a test for students. The words accompanied by an asterisk (*), however, should be familiar to the students before they begin the module.

alcoholism: the addiction to regular consumption of alcohol with deleterious physical and behavioral effects.

***allele:** an alternative form of a gene; any one of several mutational forms of a gene.

***autosome:** any nuclear chromosome other than the X and Y chromosomes.

BMD: Beckers muscular dystrophy.

carrier: an individual heterozygous for the gene for a recessive trait.

cDNA: complementary DNA; a DNA copy of mRNA.

CF: cystic fibrosis.

chromosome banding: a technique for staining chromosomes so that bands appear in a unique position according to the particular chromosome.

cloned DNA DNA that has been isolated and inserted into a vector such as a plasmid or a yeast artificial chromosome.

***codon:** a sequence of three nucleotides in mRNA that specifies an amino acid.

conservative change: an amino acid change that does not affect significantly the function of the protein.

cytogenetics: the study of chromosomes.

degenerate codon: a codon that specifies the same amino acid as another codon.

***deletion:** the loss of a piece of chromosome with the genes it carries.

***diagnosis:** the act or process of identifying or determining the nature of a disease by examination.

***discrimination:** an act based on prejudice.

DMD: Duchenne muscular dystrophy.

DOE: Department of Energy.

ethics: The branch of philosophy that considers questions of good or bad and right or wrong.

***exons:** the DNA segments of a gene that ultimately are incorporated into mRNA.

gel electrophoresis: the process by which nucleic acids (DNA or RNA) or proteins are separated by size according to movement of the charged molecules in an electrical field.

***gene:** the hereditary unit of DNA that occupies a certain spot on a chromosome, has a specific effect on the phenotype, and can mutate to various allelic forms.

gene mapping: the process of determining the location of genes at specific sites on chromosomes.

gene therapy: insertion of functional genes into cells that lack those genes.

genetic counseling: the educational process that assists individuals, couples, or families with decisions related to genetic disorders with which they may be affected, for which they may be at risk, or for which they may be carriers.

genetic linkage map: map where the distance between markers relates to the frequency with which they are inherited together rather than being separated by recombination during meiosis.

genetic profile: details of an individual's genome.

genetic screening: the search in a population for individuals having genetic characteristics that likely will be harmful to themselves or to their descendants.

genetic testing: the search in an individual for genetic characteristics that likely will be harmful to that individual or to his or her descendants.

genome: the DNA content of an individual, which includes all 44 autosomes, the 2 sex chromosomes, and the mitochondrial DNA. For the HGP, one each of the different chromosomes—22 autosomes, plus X, plus Y, plus a mitochondrial chromosome.

***genotype:** genetic constitution of an organism.

***heterozygous:** having two alleles that are different for a given gene.

HGP: Human Genome Project.

HHMI: Howard Hughes Medical Institute.

***homozygous:** having two alleles that are identical for a given gene.

HUGO: Human Genome Organization.

hybridization: the pairing of a single-stranded, labeled probe (usually DNA) to its complementary sequence.

imprinting: a chemical modification that marks which allele of a gene came from the mother and which came from the father.

***in situ* hybridization:** hybridization of a labeled probe to its complementary sequence within intact, banded chromosomes.

***introns:** the DNA segments of a gene, between exons, that are transcribed into nuclear RNA, but are removed in the subsequent processing into mRNA.

***karyotype:** the arrangement of a set of photographed, banded chromosomes in order.

linkage analysis: the tracking of a genetic trait by following the inheritance of a closely associated gene or DNA marker.

marker: trait, gene, or fragment of DNA that can be identified on a genetic or physical map.

missense mutation: a change in the base sequence of a gene that alters or eliminates a protein.

***mRNA:** messenger RNA.

multifactorial: a characteristic influenced in its expression by many factors, both genetic and environmental.

NIH: National Institutes of Health.

nonsense mutation: a mutation in which a codon is changed to a stop codon, resulting in a truncated protein product.

northern analysis: hybridization of a labeled probe to RNA that is bound to a nylon membrane.

PCR: polymerase chain reaction; a technique that uses double-stranded DNA and two primers as templates for numerous duplications. At each round of duplication the amount of DNA product doubles.

pedigree: a record of the heredity of a particular trait through many generations of a family.

***phenotype:** observable characteristics of an organism produced by the organism's genotype interacting with the environment.

physical map: map where the distance between markers is the actual distance, such as the number of base pairs.

PKU: phenylketonuria.

plasmid: circular, bacterial DNA into which a fragment of DNA from another organism can be inserted.

predisposition: to have a tendency or inclination toward something in advance.

presymptomatic diagnosis: diagnosis of a genetic

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disorder before the appearance of symptoms.

primer: nucleotides used in the polymerase chain reaction to initiate DNA synthesis at a particular location.

***probe:** single-stranded DNA tagged with radioactivity or some other label, which hybridizes to its complementary sequence.

prognosis: prediction of the course and probable outcome of a disease.

public policy: a set of action guidelines or rules that result from the actions or lack of actions of governmental entities.

***recombination:** the natural process of breaking and rejoining DNA strands to produce new combinations of genes and, thus, generate genetic variation.

somatic-cell hybrid: hybrid cell line derived from two different species; contains a complete chromosomal complement of one species and a partial chromosomal complement of the other.

Southern analysis: hybridization of a labeled probe to DNA that is bound to a nylon membrane.

translocation: the relocation of a chromosomal segment.

triplet: in DNA, a group of three nucleotides; called codons in mRNA.

UNESCO: United Nations Educational, Scientific, and Cultural Organization.

vector: a mechanism—such as a bacterial plasmid, yeast, or a virus transferring DNA from one cell to another.

YAC: yeast artificial chromosome; a linear vector into which a fragment of DNA can be inserted.

zoo blot: northern analysis of mRNA from different organisms.

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Classroom Management

These four activities can be completed in 5 to 7 days, depending on the amount of time you allot for class discussion. Activity 3 contains the essay "Left Wondering" that students should read ***after*** completing Activity **3.**

STUDENT BACKGROUND

The students should have an understanding of basic genetics, in particular transcription and translation, *before* they begin this module. Students should be familiar with the words in the glossary that appear with an asterisk (*). You also may wish to review with students their understanding of genetic variation and multifactorial inheritance.

You may wish to introduce your students to ethics and public policy several days before you begin the module. Section II of the teacher narrative contains information about teaching these topics in the classroom.

TWO DAYS BEFORE YOU BEGIN

- Copy Background Information on the Human Genome Project and Activity 1. Hand out the background information and have students read the information and compile their questions and concerns as homework.
- If you plan to use pop-it beads or paper models for Activity 1, prepare the sets now.
- If you plan to use outside decision makers for Activity **3**, make the arrangements now. If you wish to combine two or more biology classes for Activity **3**, complete these arrangements as well.

ONE DAY BEFORE YOU BEGIN

- The day before you begin, have students read the information about cystic fibrosis in Activity 1. You may wish to review important genetics concepts with your students.
- Collect the student's homework from the previous day.

DAY 1, ACTIVITY 1

- This activity will take one 45-minute class period. You may wish to spend additional time for class discussion.
- Copy the student materials for Activity 2.

DAY 2, ACTIVITY *2*

- Recap the previous day's activity. Activity 2 will take one 45-minute class period, but you may wish to plan additional time for discussion.
- Copy the student materials for Activity **3**, including the essay on H D titled "Left Wondering."

DAYS 3 AND 4, ACTIVITY 3

- Recap Activity 2. Activity 3 is designed to use two 45-minute class periods. You may wish to allow more time for debate and discussion if your students are extremely interested.
- Have students read "Left Wondering" after completing the activity.
- Copy the student materials for Activity 4.
- Assign the reading in Activity 4 on alcoholism as homework.

DAY 5, ACTIVITY 4

- This activity can be completed in one class period, but you may wish to allow more time for discussion.

AFTER CONCLUDING ACTIVITY 4

- As homework, have the students review the lists they made of their feelings and concerns about the HGP at the beginning of their work on the module. Ask them to write down any new feelings or concerns they may have now that they have completed the activities and read the essay on HD. Be sure they note any changes to their original lists. You may wish to use an additional class period to discuss these concerns and feelings.

FACILITATING CLASS DISCUSSIONS*

The physical setting. The usual seating arrangement of all desks in rows facing front is unsuitable for either small-or large-group discussions. To establish a physical setting that is conducive to discussion, place chairs around small tables in a random arrangement, move desks into circles, or move stools around lab tables. If your classroom consists of bolted down desks or auditorium seats, find an open area and allow students to sit on the floor. If possible, conduct class in the cafeteria, gymnasium, conference room, student lounge, outdoors—anywhere the environment will allow face-to-face interaction.

Encouraging student participation. The following strategies will help create an atmosphere conducive to inquiry and analysis

- Establish and maintain a positive attitude by demonstrating a high level of personal enthusiasm for and familiarity with the material. Invite the student's participation and inquiry by encouraging alternative ideas and divergent views.
- Receive all responses graciously and without personal bias. Avoid rejecting any argument that is presented seriously.
- Allow students to think on their own. Avoid giving information that the students could obtain from available resources or from other members of the group.
- Allow as many students as possible to participate in the discussion. Avoid permitting a few participants to dominate the discussion.
- Respond in a positive manner to students who express opinions that disagree with your own.
- Interact with the students. Move around in the discussion group. Avoid standing at the front of the class as an authority figure.

Dealing with issues and values. This module provides an opportunity for the students to discuss, interpret, and evaluate human genetic research in light of values and ethics. The emphasis on personal values for evaluation of this knowledge may be a new experience for many students. Possibly, most critical to the flow of ideas and quality of exchange is the role and attitude of the instructor as facilitator.

Instructors sometimes feel that discussion of value issues should be reserved for older and "brighter" students. This belief is not supported by evidence and, in fact, there is much to be gained by involving students in general, introductory biology or social science courses in the analysis of issues of science, technology, and society. All students will be expected to function as citizens in a democratic society, and their school experience should provide opportunities to learn how to deal with contentious issues with civility, objectivity, and fairness. Teachers and students can and should work together to understand their own positions, to value other positions, and to respect the opinions of others. The classroom also is an appropriate place to learn how to question one's own values and those of others in ways that are productive, enlightening, and nonjudgmental.

Neutrality probably is the single most important attribute of a successful discussion facilitator. Within the framework of neutrality, certain behaviors will help members of the discussion group balance factual information with feelings

- Ask members of the group to respond to or build on each other's ideas.
- Ask questions that will help your students distinguish between those components of an idea or issue that can be answered through scientific research and those components that are a matter of values.
- Through unbiased questioning, help the participants critically examine all views presented.
- Keep the discussion relevant and moving forward by questioning or posing an appropriate problem or hypothetical situation.
- Respect all sincere contributions equally, whether you agree or disagree. This is the case for all convictions, including religious ones.
- Encourage individuals to contribute, but do not force reluctant students into the discussion.
- Respect silence between contributions. Reflective discussions often are slow. If you break the silence, the students may let you dominate the discussion.
- Avoid seeking consensus on all issues. The issues raised are multifaceted, and divergent points of view are desirable. In some cases, however, consensus on a compromise solution to a problem may be demonstrated as a powerful determinant of cooperative community action.
- Avoid praising the substance of contributions, especially points of view with which you agree. Do, however, praise the willingness of students to contribute well thought-out ideas by making such comments as "Thanks for that idea," or "Thanks for those thoughtful comments."
- Avoid asking questions that have exact answers.
- Toward the end of a discussion, ask the participants to summarize the points that they and their classmates made.

Generally, these suggestions stimulate person-to-person interaction with as little direct involvement as possible on the part of the facilitator. 'This role is important for successful discussions and individual growth in dealing with controversial ideas and issues. Initially, some participants may have difficulty responding without specific direction; however, the initial, uncomfortable phase of long silences and faltering responses is essential to stimulate each student's ability to evaluate ideas

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and values in the context of a larger problem.

Dealing with controversial issues: Genetic screening and testing, gene sequencing, prenatal diagnosis, genetic counseling, abortion, and other topics in this module or related to it potentially are controversial. How much controversy develops depends on many factors: the socio-economic climate of the community, the religious preference of your students, and the value system of your students and their parents. Most important, however, it will depend on how you handle these issues in the classroom. One method of dealing with controversy is described below.

1. Present as much information about the issue as possible. Frequently, the narrow and rigid viewpoint of the students is due to their having little, or erroneous, information about an issue.
2. Allow all opinions or feelings to be expressed.
   - Do not be a censor and forbid certain views because the views are radical or shocking.
   - It is not hard to determine when a student is saying something for its shock value, which is not constructive to the discussion. Look to see whether the other students have picked up on the inappropriate comment and ask them to respond.
3. Acknowledge each opinion in the same even-handed manner.
   - Open discussion and debate will be destroyed if the class senses you favor one group of ideas over another.
   - Your open attitude to all views will be contagious and a similar accepting climate can develop throughout the class.
   - You should make the students feel that, as long as what they say contributes to the discussion in a positive manner, they have every right to say what they feel.
4. Create an open and nonhostile atmosphere in the classroom.
   - This is not something that just happens a few minutes before the discussion begins.
   - A trusting, caring atmosphere must exist between teacher and student and among the students.
5. Emphasize that everybody—you and the students—must be open to diverse views.
   - We cannot make intelligent decisions if we close ourselves off from some viewpoints. Even if we cannot agree with or are offended by a viewpoint, it is important at least to hear it, so that we know it exists and can consider it in our decision making.
6. Keep your personal views out of the discussion.
   - Neutrality on the part of the teacher is the key to a successful discussion of controversial issues. Experts in science education recommend that teachers withhold their personal opinions from students. The position of the teacher carries with it an authority that might influence some students to accept the teacher's opinion without question—thus missing the point of the activity. There also is a danger that the discussion could slip into an indoctrination into a particular value position rather than an exploration of several positions. If your students ask what you think, respond with "My personal opinion is not important here. We want to consider your views."
   - Make sure you consider alternative points of view thoroughly enough to help your students define the relevant arguments and counter-arguments. Let the students help you promote the expression of alternative points of view.
7. Create a sense of freedom in the classroom for the students.
   - Freedom implies the responsibility to take advantage of that freedom and produce positive results for all.
   - There is a fine line between freedom and license. In general, freedom is a positive influence, and license usually has some negative results.
8. Finally, your students should know that you respect them for who they are, not for what they might say under stressful conditions.
   - If they feel they must respond in a certain manner to gain your approval, you do not have an open discussion.
   - If they know that you are accepting of what they say because of who they are, discussion should be open and valuable.

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Background Information

Activity 1

DNA Sequences

Read the following information about the Human Genome Project (HGP) and write down your feelings about the HGP on a separate sheet of paper. Your teacher will ask you to refer to your record after Activity **4**. If you wish, discuss this information and the HGP in general with your family and friends.

The Human Genome Project:
Mapping and sequencing Human Genes

Suppose that it becomes possible for geneticists to identify the genes that predispose certain people to heart disease, or those genes that will cause individuals to become blind as they age. If you knew what genes you have and what genetic disorders they might cause, would it affect your choice of careers? Would it affect your decision to marry or begin a family? Suppose your potential employer also had access to your genetic information. Could the fact that an employer had knowledge about your genetic information result in job discrimination? If you knew that some genes in your unborn baby might cause him or her to suffer from impaired mental or physical performance, would this affect your decision to carry the fetus to term? These are some of the issues raised by the Human Genome Project, or HGP. Decisions such as these likely will confront society as the HGP generates information on the content of the human genome.

Human cells that have nuclei (except for eggs and sperm) normally contain 22 pairs of autosomes, plus two sex chromosomes (XX or XY). These cells also contain mitochondria, the powerhouses of the cell. Each mitochondrion has a small circular chromosome. The total genome for any individual includes all of these components.

The primary goal the HGP is to uncover the genetic information contained in the human chromosomes. For the purposes of the HGP, ***genome*** means one each of the different chromosomes—22 autosomes (not the diploid number of 44), plus X plus Y, plus a mitochondrial chromosome. This genetic material includes approximately 3 billion base pairs of DNA, containing 50,00-100,00 genes. Researchers will map each chromosome and then attempt to determine the DNA sequence of each gene. The HGP will not provide the DNA sequence from one individual, but rather a collection of sequences from different people. The knowledge and technology that result from the HGP eventually may allow researchers to describe the total genome of ***Homo sapiens.***

The project, which is funded in the United States primarily by the Department of Energy and the National Institutes of Health will take about 15 years and will cost approximately $3 billion to complete. About six other countries also are involved in the HGP. In addition to groups that address the science and data storage issues, there are groups that address social, ethical, and legal implications of genome research.

WHAT MIGHT WE LEARN?

Biologists already have some ideas about the possible results of the HGP, although there may be surprises as well. Geneticists are attempting to isolate genes responsible for certain genetic disorders. Recent successes include the identification of the genes responsible for Duchenne muscular dystrophy (DMD) and cystic fibrosis (CF), two serious genetic disorders. By isolating such genes, biologists can learn about the structure of the gene's corresponding protein and the cause of the disorder. This knowledge could lead to better medical management of such diseases.

Genome information also can indicate whether an individual is likely to develop a disease in the future. For example, if the gene for Huntington disease is present, it is a near certainty that symptoms eventually will occur. The HGP also may help predict which individuals have an increased susceptibility to disorders such as heart disease, cancer, or diabetes. Because these disorders result from complex interactions between genes and the environment, there is no certainty that symptoms will develop. The risk, however, is greater for certain individuals than for the general population. Biologists also are hopeful that the HGP will shed light on basic biological questions such as human evolution, development, and regulation.

In addition, the HGP also is responsible for the development of new technologies that will have an impact outside the HGP itself. One such technology is the development of sophisticated data management systems and computers to handle the enormous amount of information generated by the HGP.

WHAT ARE SOME POTENTIAL PROBLEMS?

As with many large projects, critics have expressed several concerns about the HGP. One major criticism is that the high cost cannot be justified because we will not learn very much about what it is to be human simply by sequencing all the genes in the human genome. We also must take into account the numerous influences of the environment. Other critics maintain that funding such a large project takes scarce resources away from individual researchers who may study more efficiently certain areas of particular scientific or medical interest. In addition, allocating funds for research on genetic disorders takes money away from programs that help relieve other causes of ill health. Perhaps the genome money would be better spent, for example, on prenatal care for all pregnant women, on nutrition and basic health care for children living in poverty, or on **AIDS** research.

Some critics suggest that the ability to diagnose a genetic disorder before any treatment is available does more harm than good because it creates anxiety and frustration. Even in the absence of new treatments, however, the HGP may make diagnosis possible before the onset of symptoms and, thus, make the management of the disorder more effective. In addition, improved knowledge of one's genetic background can provide couples with a broader range of options for family planning.

Other critics object to the HGP because they believe we do not have the ability to regulate the ultimate outcomes. Some critics do not feel that just because scientists *can* do this science, they *ought* to. These critics point to the development of atomic weapons and argue that the science that led to their development caused far more problems than it resolved.

It is possible that the HGP will allow the determination of personal genetic profiles—an individual's genome data as opposed to genome data that are reflective of the general human population. There are many unanswered questions about the use of such individual data. Who will have access to such personal genome data? Should employers or insurance companies be permitted to use the data to discriminate against individuals? How can we ensure the privacy of the very large data bases to which many individuals and organizations have access? Should information such as disease-carrier status or paternity information be provided to family members who did not seek the information themselves?

Some critics feel that the genome project gives too much emphasis to the genetic component of human characteristics while ignoring the environmental components. They worry that this emphasis will cause society to blame people for having "bad" genes while failing to improve environmental and societal factors that might help such individuals escape the effects of those genes. What do you think about the HGP?

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Activity 1
DNA Sequences
Copymaster

One possible result of the Human Genome Project (HGP) is the ability to identify differences in the DNA of people who have inherited genetic disorders. This is accomplished by sequencing the bases in DNA. DNA is a linear molecule composed of four different bases: adenine, cytosine, thymine, and guanine (abbreviated A,C, T, and G). The information contained in the DNA molecule is encoded in these four bases.

These bases are "read" in groups of three, called triplets. These triplets code for specific amino acids, which form specific proteins. Mutations in the base sequence can change the resulting protein structure. This can happen if the mutation alters the amino acid specified by a triplet or if it alters the sequence of bases in a triplet by deleting or adding a base. By sequencing the DNA bases from different pebple (some who have and some who do not have a genetic disorder), geneticists can look for differences in the sequence that might cause the disorder.

The DNA base sequence is the most detailed map of our inherited material. Other types of maps of the inherited material are less detailed than the base sequence map, but they help geneticists find the particular segment of DNA they want to study. This process is similar to using a map of the world to locate the United States, then using the map of the United States to locate Massachusetts, using a map of Massachusetts to locate Boston, where 563,000 people live, and finally using a street map of Boston to locate the street address where a specific family lives (see Figure 1.1).

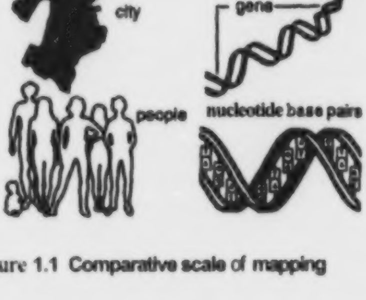

Figure 1.1 Comparative scale of mapping

WHAT IS CYSTIC FIBROSIS?

Cystic fibrosis (CF) is an autosomal recessive, inherited genetic disorder that affects mainly Caucasians. It is rare in Blacks and Orientals. CF affects approximately 30,000 children and young adults in the United States and it occurs in about one in every 2,500 newborns. Approximately 1,400 new cases are diagnosed each year, usually within the first three years of life. The basic symptoms of CF include serious digestive and respiratory problems and extremely salty sweat. These symptoms result from the inability of the exocrine glands to reabsorb chlorine and sodium. As a result, thick, sticky secretions clog up the tubules in the linings of the internal organs and cause irreversible damage to various organ systems.

Clogged bile ducts can cause cirrhosis of the liver and pancreatic tubules can rupture and spill out digestive enzymes that can lead to the formation of fibrous tissue. Because these pancreatic enzymes do not reach the intestines, the digestion and reabsorption of proteins and fats is incomplete. The accumulation of thick mucus in the small airways of the lungs results in chronic coughing, impaired breathing, and a greatly increased (and often fatal) susceptibility to bacterial infections. Because the ducts of the sweat and salivary glands do not reabsorb chlorine and sodium efficiently, individuals who have CF have extremely salty sweat—about five times as salty as that of unaffected individuals.

CF symptoms can become more severe with age. For example, in older patients who have CF, respiratory problems are more severe, but the digestive symptoms can be controlled by taking replacement pancreatic enzymes. Adults tend to develop nasal and cardiac problems, mild diabetes, and emotional difficulties, and they also may suffer from massive bleeding from the bronchial tubes. More than 95 percent of males who have CF are sterile because of damaged tubules in the testes, and females who have CF may experience reduced fertility as well.

THE CF GENE

To identify the CF gene, geneticists used a ***linkage map*** to determine which chromosome contains the CF gene. They looked for markers (fragments of identifiable DNA) on specific chromosomes by comparing the DNA of CF-affected individuals to that of their parents, relatives, and individuals from other families. Eventually, they determined that chromosome 7 carries the normal gene that is mutated in CF, and they established the gene's general location between the known markers.

Although a linkage map is a powerful tool for narrowing the search for genes, it is not sufficient for actually isolating a gene and removing it for study. For this, geneticists use a ***physical map***. A physical map provides the actual distance between landmarks on the chromosomes. The distance between sites on a chromosome is measured by physical length, for example, the number of base pairs. The ultimate physical map would be the exact order of the 3 billion bases that constitute the human genome. Scientists used a variety of techniques to help determine the physical map of the CF region. By DNA "walking" (using partly overlapping DNA fragments to come closer to the target gene) or DNA "jumping" (using a circular piece of labeled DNA to bridge long sections of DNA), scientists then created a chain of overlapping segments of DNA in the space between the flanking markers of the CF gene. The physical map of the region where the CF gene is located indicated to the researchers the location of each piece of DNA in the region. They then examined the base sequences of these individual pieces of DNA for a difference that might cause the genetic abnormality in CF. At least 70 percent of individuals who have CF share a common mutation in their sequence of bases.

When the CF gene was discovered in August 1989, the pace of CF research greatly accelerated. In 1990, scientists successfully copied the unaffected CF allele and added it to CF cells (in a laboratory experiment, *not* in humans), thus correcting the defective CF cells. This was the first step in "gene therapy" for CF. The second major step toward gene therapy now is underway—CF researchers are refining new gene transfer technologies that may lead to more effective treatment or a cure.

In this activity, you will examine the DNA sequences in a small portion of the CF gene from six students and determine a few types of mutations that can cause CF.

PROCEDURE

1. Geneticists use several methods to determine the base sequence in a piece of DNA. In one common method, they use radioactivity to mark different DNA bases and examine the radioactive bases on a piece of

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photographic film. Each row across the film is a position for a DNA base. A dark band in the column for a specific base tells the geneticist which base is in that position. This is similar to a map that tells you who (A,C, G, or T) lives at which street address (1, 2, 3, and so on). Examine Figure 1.2 and read the positions from top to bottom. What is the base sequence for the 10 positions in the box? This procedure allows geneticists to read many bases of DNA. They also can compare the base sequences for the same gene in many different people.

2. Figure 1.3 represents the bases present in a small part of the gene for CF (from one chromosome) for six different high school students. The entire CF gene contains about 250,000 bases—far too many to list on this page. Working in teams of three, use the worksheets your teacher provides to list the sequences of one strand of DNA from the maternal chromosome for the individuals listed at the top of the worksheet. The corresponding base sequences from the paternal chromosome appear alongside each individual's sequences on the worksheet. (Remember, chromosomes come in pairs—one from the mother [maternal], the other from the father [paternal].)

3. Exchange worksheets with one of your partners and check each other's lists.

4. With your partners, examine the base sequences of both chromosomes from Norma, Karen, and Josina, who do not have CF. Circle any differences. (Later you will discuss how a person can have a difference in his or her DNA sequence and not have a genetic disorder.)

5. Examine the base sequences for Leah, Martin, and Richard. Circle any differences between their sequences and those of the unaffected individuals.

6. What is especially different about the DNA sequence for Richard? Propose a hypothesis to explain your observation. (Hint: Line up the base sequences from Richard and Martin, beginning with position 24 and working backwards until you come to a discrepancy.)

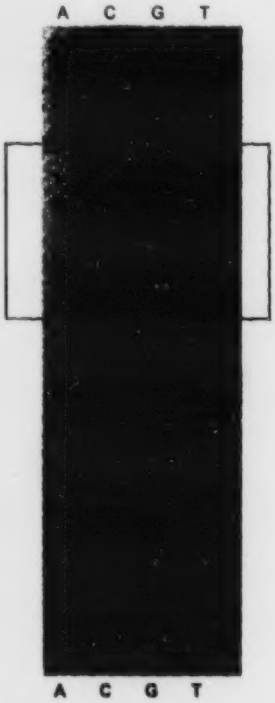

Figure 1.2 **An autoradiograph**—a method to determine base sequences

7. Recall that the base sequence, in triplets, codes for specific amino acids. In steps 2-5, you discovered that some differences in the base sequence for a gene cause a genetic disorder (such as CF), whereas other differences have no effect. A question left unanswered was: How can a person have a difference in his or her base sequence and yet not have a genetic disorder? Propose a hypothesis to answer this question.

8. Use Figure 1.4 to determine the amino acids coded for in the eight DNA triplets you listed on the worksheet. Record the appropriate amino acids in the proper columns on the worksheet.

9. The amino acid sequence determines the protein's composition. Compare the protein formed by this portion of the base sequences for Norma, Karen, and Josina.

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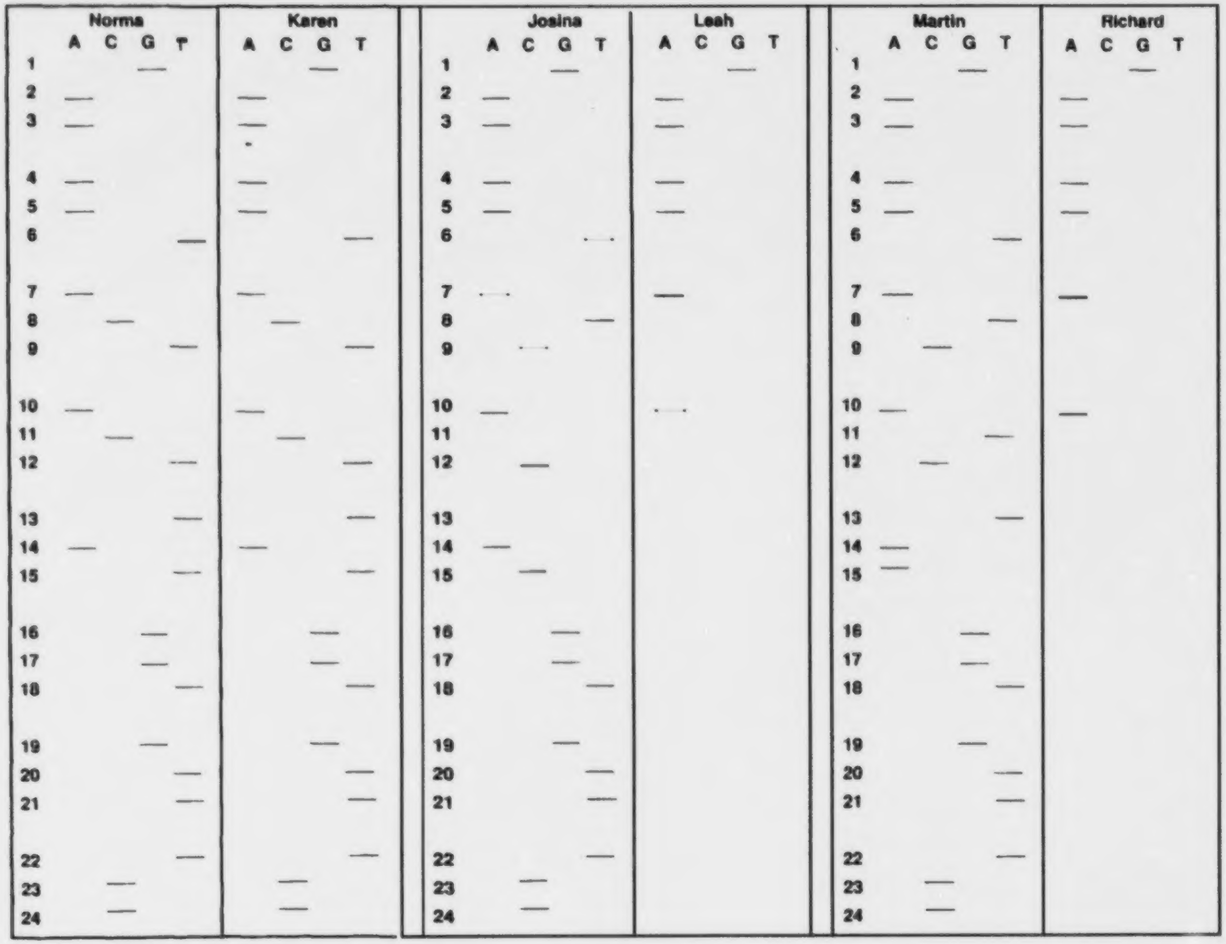
Norma
Karen
Josina
Leah
Martin
Richard
A C G T
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24

Figure 1.3 DNA sequences

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What do you find? This should help explain why some people with different base sequences are unaffected by genetic disorders.

10. Examine the amino acid sequence for the proteins of Leah, Martin, and Richard. What causes CF in these people?

QUESTIONS FOR DISCUSSION

1. Cystic fibrosis **is** an autosomal recessive disorder. That means that affected individuals have inherited an abnormal allele from each parent. Would a person who has a sequence like Leah's and a second sequence like Norma's have CF?
2. Assume that a person has one allele with the sequence shown for Leah and a second allele with the sequence shown for Richard. Would the person have CF? Explain your answer.
3. A person can have the same sequence of DNA for the **24** bases as does Josina and still have **CF**. Propose a hypothesis to explain this. How might you test your hypothesis? Remember that the **24** bases of the DNA sequence you have examined represent only a small fraction of the DNA (approximately 250,000 bases) found in the CF gene.
4. Read the advertisement for CF testing and the screening policy statement from The National Institutes of Health your teacher provides. Discuss the relevant implications of cystic fibrosis testing and genetic screening in general.

First Nucleotide	**Second Nucleotide**				**Third Nucleotide**
	T	**C**	**A**	**G**	
T	TTT } phenylalanine	TCT } serine	tyrosine	TGT } cysteine	T
	TTC	TCC		TGC	C
	} leucine	TCA	TAA } stop	TGA stop	A
		TCG	TAG	TGG tryptophan	G
C	CTT } leucine	CCT } proline	CAC } histidine	CGT } arginine	T
	CTC	CCC		CGC	C
	CTA	CCA	} glutamine	CGA	A
	CTG	CCG		CGG	G
A	ATT } isoleucine	ACT } threonine	AAT } asparagine	AGT } serine	T
	ATC	ACC	AAC	AGC	C
	ATA	ACA	AAA	AGA } arginine	A
	ATG) methionine/start	ACG	AAG } lysine	AGG	G
G	GTT } valine	GCT } alanine	GAT } aspartic acid	GGT } glycine	T
	GTC	GCC	GAC	GGC	C
	GTA	GCA	GAA } glutamic acid	GGA	A
	GTG	GCG	GAG	GGG	G

Figure 1.4 The genetic code

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Statement from the National Institutes of Health Workshop on Population Screening for the Cystic Fibrosis Gene

Cystic fibrosis is an autosomal recessive genetic disorder clinically characterized by chronic lung disease and pancreatic insufficiency. Median survival is 25 years, with an increasing number of patients surviving into their 30s. This disease affects about 1 in 2,500 persons of European ancestry. It is less frequent among black and Hispanic Americans and is rare in Asians. One in 25 persons of European ancestry is a carrier, having one normal and one abnormal cystic fibrosis gene.

The cloning of the cystic fibrosis gene and identification of the most common mutation in the gene were major advances in medical genetics. These breakthroughs provide a basis for understanding the patho-physiology of the disease and offer the hope the improved therapy can be developed. In addition, there are immediate implications for the identification of carriers of a mutant cystic fibrosis gene.

Currently, DNA analysis can identify a single mutation in the cystic fibrosis gene that is present in 70 to 75 percent of carriers of European ancestry*. Many additional mutations producing cystic fibrosis, currently about 20, have been identified. The identification of multiple individually rare mutations rather than a small number of common mutations will make carrier testing more difficult. The inability to detect all carriers creates complexities for the use of carrier testing at this time.

A major question is whether population-based screening for cystic fibrosis carriers could or should be implemented at present. Population-based screening implies offering a program of carrier testing, with appropriate informed consent and genetic counseling, to potentially millions of healthy people. The purpose of such screening would be to allow people to make more informed reproductive decisions with regard to the risk of cystic fibrosis in their offspring.

Unlike testing in the general population, testing for carriers in families in which the disease has occurred is nearly 100 percent informative. This is because carrier testing can be performed with linkage analysis in addition to mutation analysis when there is a DNA sample available from an infected person in a family. Therefore, testing should be offered to all individuals and couples with a family history of cystic fibrosis. This makes it more important than ever for providers of medical care to obtain family histories, particularly for patients of reproductive age.

In contrast, for a number of reasons there is a consensus that under the current circumstances population-based screening should not be recommended for individuals and couples with a negative family history. First among these reasons is the fact that currently the test will detect only about 70 to 75 percent of carriers. Therefore, only about half the couples at risk can be identified. Second, the frequency of the disease and the different mutations vary according to racial and ethnic background, so that important laboratory and counseling modifications would be required in different populations. Third, there are substantial limitations on the ability to educate people regarding the use of an imperfect test. Fourth, without more definitive tests, about 1 in 15 couples—those in which one partner has a positive test and the other has a negative test—would be left at increased risk (approximately 1 in 500) of bearing a child with cystic fibrosis.

These difficulties would be substantially reduced if testing could detect at least 90 to 95 percent of carriers. There is a consensus that population-based screening for carriers could be offered to all persons of reproductive age if a 95 percent level of carrier detection were achieved. The offering of population-based screening would still require that substantial educational and counseling guidelines be satisfied. Benefits and risks of population-based screening and its feasibility are uncertain if this level of carrier detection cannot be achieved. When people without a family history of cystic fibrosis request testing, the physician should explain the risks and benefits of the test, either directly or through a center for genetic counseling.

Regardless of when or whether population-based screening becomes widespread, there is a consensus on a number of screening guidelines. First, screening should be voluntary, and confidentiality must be assured. Second, screening requires informed consent. Pretest educational materials should explain the hazards (for example, psychosocial effects and the loss of insurability) and benefits of choosing to be tested or choosing not to be tested. Third, providers of screening services have the obligation to ensure that adequate education and counseling are included in the program. Fourth, quality control of all aspects of the laboratory testing, including systematic proficiency testing, is required and should be implemented as soon as possible. An finally, there should be equal access to testing.

In view of the importance of the voluntary nature of screening and anticipated rapid changes in current information, legislative action to require cystic fibrosis screening is undesirable at present. There is consensus, however, that cystic fibrosis carriers should not be discriminated against with regard to insurability or employment. If evidence of discrimination emerges, corrective legislative action should be considered.

When population-based screening becomes available, who should be offered the testing and in what setting? The most appropriate group for population-based screening comprises those of reproductive age. Although it is recognized that testing will often be provided to couples during pregnancy, it is preferable to offer screening before conception. Preconception testing offers a couple that has a one-in-four risk of having an affected child a broader range of reproductive options.

The optimal setting for carrier testing is through primary health care providers. Community- based screening programs provide an alternative setting. At present, newborn screening primarily to detect carriers is inappropriate, as are screening programs directed at children below reproductive age.

Education of the lay community and health care providers concerning the disease, its genetic transmission, and carrier testing is an important goal. Both traditional and innovative methods should be used for community education. Education regarding carrier testing for cystic fibrosis could have a major effect on the level of genetic knowledge in the population.

In addition to obtaining informed consent, providers of carrier screening must ensure the availability of appropriate genetic counseling. Those with a negative carrier test should require minimal counseling but should be reminded of the limitations of the test. Those identified as carriers should be informed of the personal and family implications of their status.

Group and individual counseling services should be available. After specialized training, physicians, nurses, social workers, and other health care personnel could provide much of this counseling service. A range of teaching devices to be shared with family members should be developed and evaluated as an adjunct to counseling.

Couples found to have a one-in-four risk require comprehensive individual genetic counseling by a qualified professional. This counseling should be nondirective and should help the couple to make reproductive decisions consonant with their own beliefs. Educational materials that present a range of views and options should be developed.

Pilot programs investigating research questions in the delivery of population-based screening for cystic fibrosis carriers are urgently needed. These programs should address clearly defined questions, including the effectiveness of educational materials, the level of utilization of screening, laboratory aspects, counseling issues, costs, and the beneficial and deleterious effects of screening. One important issue is to determine the effect on couples who are at increased risk because only one partner has an identified cystic fibrosis mutation. Pilot programs should examine alternative models of delivery that could be generalized. Federal funds are critically needed to carry out these programs.

The development of carrier-screening programs for cystic fibrosis should not detract from the current scientific efforts to improve the treatment of this disease. Currently, about 30,000 Americans have cystic fibrosis, and additional children with the disease are born every day. The ability to identify couples at risk will continue to be important even if more successful treatments are developed.

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'Currently, screening tests can detect between 90 and 95 percent of the known mutations in CF carriers of European ancestry. How might this ability affect the NIH position on population screening for CF?

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Now ... A Test to Identify Greater Than 90%of *Cystic* Fibrosis Carriers*

By Concurrent Detection of the Phe508 Deletion
Plus Nine Other Major Mutations

- Rapid Turnaround
- Competitive Pricing
- CF Linkage Testing If Needed
- To Receive Our Free Prepaid Mailing Package, Please Call 1-800-356-4566 or FAX 617-891-5062 Immediately

* As applied to individuals of northern European ancestry – other ethnic groups may differ

From Your Quality Source of Advanced Genetic Testing

Diagnostic Services Division

Collaborative Research Incorporated

1365 Main Street, Waltham, MA 02154

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Test Background

Cystic fibrosis is the most frequent recessive genetic disease among Caucasians. It strikes approximately 1 in every 2500 newborns. About 1 in every 25 Caucasians is a silent carrier of a defective gene. The cystic fibrosis gene has been cloned and additional research has established that a majority of the carriers of cystic fibrosis have a single mutation which accounts for 70% of the carriers. Our current assay tests for the presence of this mutation (known as the Phenylalanine 508 Deletion) and nine other major mutations (621+1G➤T, ΔI507, 1717-1G➤A, G542X, G551D, R553X, R560T, W1282X, and N1303K). A positive result for the presence of any of these mutations means that the patient is a carrier of a cystic fibrosis gene and that other family members should consider being tested.

It should be noted that cystic fibrosis can be caused by other alleles which have not as yet been fully characterized. Therefore, a negative result for the presence of any of these ten mutations, while significantly reducing an individual's risk of being a carrier, does not rule out the possibility of the individual being a carrier of another mutation. As other major mutations become known, Collaborative Research will incorporate them into our own assay.

Who Will Benefit?

About 8 million Americans carry a CF gene. Until 1990, the DNA-based test for CF carrier detection was a cumbersome linkage test which could only be used in a family study for those families in which there was already a history of the disease. With the cloning of the CF gene and the identification of the Phenylalanine 508 Deletion, testing of *individuals* became possible. In March 1990, a special NIH Workshop on Population Screening for the Cystic Fibrosis Gene recommended that the Phenylalanine 508 Deletion test "be offered to all individuals and couples with a family history of cystic fibrosis':

The members of that workshop also suggested that testing of individuals with no family history of the disease should not begin until, among other things, the test could detect "at least 90 to 95 percent of carriers': This *new* CF carrier test satisfies that requirement for Caucasians of northern European ancestry.

For those individuals who are found not to carry any of these ten mutations, the negative result will, in effect, reduce their chance of being a CF carrier to only about 1 in 250. Thus, this new test may be of interest to ANYONE who has not yet completed their reproductive plans.

Genetic Counseling

This new test detects only carriers with one of these ten mutations. A negative result does not rule out the possibility that an individual is a CF carrier. Thus, it is important that health care providers, who refer samples for testing, be able to counsel the patient about the test results or refer the patient for such genetic counseling.

An ad hoc committee composed of members of the National Societv of Genetic Counselors used a grant from Collaborative Research, Inc. to prepare a booklet entitled "Genetic Testing for Cystic Fibrosis: A Handbook for Professionals". These booklets are available free of charge from either Collaborative Research, Inc. or the National Society of Genetic Counselors. In addition, Collaborative Research, Inc. has available a genetic specialist who can assist health care providers with the interpretation of test results.

Company Qualifications

As a pioneer in the field of DNA probe development, genome mapping, and genetic testing, Collaborative Research was initially involved in establishing linkage with the CF gene and localizing it on chromosome 7. We are committed to using our extensive background and depth of experience in the diagnostic use of DNA probes to provide the most advanced, state-of-the-art cystic fibrosis carrier testing possible.

Test Methodology

Six pairs of synthetic oligonucleotide primers are used to amplify the patient's DNA by the GeneAmp™ Polymerase Chain Reaction (PCR) process? The primers bracket the regions in which the ten mutations occur and were designed such that the presence of any of these mutations results in a smaller DNA product or a DNA product which has lost or gained a restriction site. These changes are detected visually after challenging the amplified DNA products with a restriction enzyme(s), fractionating the resulting DNA fragments by gel electrophoresis, and staining the gel with ethidium bromide.

This assay allows a clear distinction between normal, carrier, and affected individuals who carry any combination of these ten mutations.

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* The GeneAmp™ Polymerase Cham Reaction (PCR) process is covered by U.S. patents 4,683,195 and 4,683,202 issued to Cetus Corporation and licensed to Collaborative Research, Inc.

Ordering Information

Specimen Requirements: 1x8cc tube of whole blood, collected in an ACD tube. Such a specimen can be shipped at room temperature by Express Mail to our laboratory. We supply all necessary tubes and shipping materials at no charge, upon request.

Other Requirements: Under New York state regulations, samples submitted from that state must be accompanied by a signed CRI consent form which is available upon request.

Reporting of Results: The laboratory will communicate test results, in confidence, only to the reference laboratory, physician, or genetic counselor who has ordered the test.

Price: $223.00/Specimen

Diagnostic Services Division

Collaborative Research Incorporated

1365 Main Street, *Waltham*, MA 02154 800-356-4566 *or inside* MA 617-894-5807

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Activity 1 Worksheet A Base Sequences

	Norma			Karen		
Position #	Chromosome 7 from Mother	Amino acid	Chromosome 7 from Father	Chromosome 7 from Mother	Amino acid	Chromosome 7 from Father
1			G			G
2			A			A
3			A			A
4			A			A
5			A			A
6			T			T
7			A			A
8			T			T
9			C			C
10			A			A
11			T			T
12			C			C
13			T			T
14			A			A
15			T			C
16			G			G
17			G			G
18			T			T
19			G			G
20			T			T
21			T			T
22			T			T
23			C			C
24			C			C

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Activity 1 Worksheet B Base Sequences

	Josina			Leah		
Position #	**Chromosome 7 from Mother**	**Amino acid**	**Chromosome 7 from Father**	**Chromosome 7 from Mother**	**Amino acid**	**Chromosome 7 from Father**
1			G			G
2			A			A
3			A			A
4			A			A
5			A			A
6			T			T
7			A			A
8			T			T
9			C			C
10			A			A
11			T			T
12			C			C
13			T			T
14			A			C
15			C			T
16			G			G
17			G			G
18			T			T
19			G			G
20			T			T
21			T			T
22			T			T
23			C			C
24			C			C

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Activity 1 Worksheet C Base Sequences

		Martin			Richard	
Position #	Chromosome 7 from Mother	Amino acid	Chromosome 7 from Father	Chromosome 7 from Mother	Amino acid	Chromosome 7 from Father
1			G			G
2			A			A
3			A			A
4			A			A
5			A			A
6			T			T
7			A			A
8			T			T
9			C			C
10			A			A
11			T			T
12			C			C
13			T			G
14			A			G
15			A			T
16			G			G
17			G			T
18			T			T
19			G			T
20			T			C
21			T			C
22			T			
23			C			
24			C			

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Activity 2
Do Genes Determine Our Future?
Copymaster

The knowledge and technologies arising from the Human Genome Project probably will allow biologists to determine the genes in individual genomes (46 chromosomes plus the mitochondrial DNA). This activity assumes that the details of an individual genome—a genetic profile—will be a part of a person's medical record in the future. Although the exact nature of the information the HGP will generate is unknown, it is possible we will discover genes that affect traits such as height and body build as well as genes that predispose us to disorders such as heart disease. Who will have access to this information? How will an individual use the information? Could the information provide guidance in selecting a suitable career? Would genetic information provide a complex picture of the likelihood of success in a career? Might the information become an unfair factor in the choice of a career?

PROCEDURE

1. Table **2.1** lists small portions of 10 genetic profiles. Each letter represents a gene, a unit of genetic information, which is formed by a specific sequence of the bases adenine, cytosine, guanine, and thymine. (Upper and lower case letters represent the different alleles—or forms—of each gene.)

   Your teacher will assign you a particular number that becomes "you." Form a group with classmates who were assigned the same genetic profile. Work together to determine the genotype of your profile. To do this, record in your data book the number of alleles (upper and lower case letters) present for each gene in your profile. The sequences of letters represent maps of chromosome pairs that carry genes for characteristics that might be important in different types of careers. (A slash (/) indicates the end of a chromosome.)

2. Check your genotype and then use it to determine your phenotype—your potential for each trait listed in Table 2.2. (Note that most of the traits involve two or three genes, each having two alleles.) Record these potentials in your data book.

3. Using the career examples listed in Table 2.3, speculate about the types of careers that would be particularly suitable for your profile and the types that would be unsuitable. List suitable and unsuitable careers for your genetic profile. Also list the reasons for deciding which careers were best suited and which were least suited to your profile.

Table 2.1 Genetic Profiles

1	(paternalgenes)	i	S	C	P	M	B	h/I	v	b	c	c/M	i	P	P	v	S
	(maternalgenes)	i	S	c	p	M	B	H/i	V	b	c	c/M	I	P	P	v	S
2	(paternalgenes)	I	S	c	P	M	B	h/i	v	B	c	c/m	i	p	P	v	s
	(maternalgenes)	i	S	C	P	M	B	h/i	v	B	c	c/m	i	p	p	v	S
3	(paternalgenes)	I	S	c	P	m	B	h/I	v	B	c	c/m	I	P	P	v	S
	(maternalgenes)	i	S	c	P	m	b	h/i	v	b	C	c/m	i	P	P	v	S
4	(paternalgenes)	i	S	c	P	M	b	h/i	v	b	c	C/m	i	p	p	v	s
	(maternalgenes)	i	S	C	P	m	b	h/I	v	b	C	c/m	i	p	p	v	s
5	(paternalgenes)	I	s	c	p	m	b	h/i	V	b	c	c/m	I	p	p	V	s
	(maternalgenes)	I	s	c	P	M	B	h/I	v	B	c	c/M	I	P	P	v	s
6	(paternalgenes)	I	s	C	p	M	B	h/I	V	B	C	C/M	I	p	p	v	s
	(maternalgenes)	I	s	C	p	M	b	h/I	V	B	c	C/M	I	p	p	V	s
7	(paternalgenes)	i	S	C	P	M	b	h/i	v	b	c	c/M	i	P	P	V	s
	(maternalgenes)	I	S	c	P	m	b	h/I	v	b	C	C/M	I	P	p	V	S
8	(paternalgenes)	I	S	c	p	m	b	h/i	v	b	C	C/m	i	P	P	v	S
	(maternalgenes)	I	S	c	P	m	b	h/i	V	b	C	C/m	i	P	P	v	S
9	(paternalgenes)	I	S	c	p	M	B	h/i	V	B	c	c/M	I	p	p	V	s
	(maternalgenes)	I	S	c	p	m	B	h/i	V	B	c	c/m	I	P	p	V	s
10	(paternalgenes)	I	s	C	P	M	B	h/I	V	b	C	C/M	I	P	P	V	s
	(maternalgenes)	I	s	C	P	M	b	h/I	v	b	C	C/M	I	P	P	V	S

4. Present your profile description and possible career choices to the class. Explain why you selected those particular careers and ruled out others.

QUESTIONS FOR DISCUSSION

1. What were the major reasons you gave to disqualify certain profiles from certain careers?
2. Would individuals with different genotypes be suited for some of the same careers? Give examples.
3. If your genotype was not very well suited for what you wanted to do, what might you do about it?
4. What factors are important in determining how successful a person will be in his or her career?
5. How might your gender affect your suitability for particular careers?
6. If your genotype at birth showed a moderate ability for sports, do you think your parents would encourage you to become a professional athlete?
7. How might your family's economic status affect your genotype and the resulting phenotype?
8. If your genetic profile showed a high risk for heart disease, how could you use this information to determine whether you eat more beef or more fruits and vegetables?
9. How might having a genetic profile be helpful?
10. Assume that 20 years from now your ge-

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Table 2.2 Potential for Traits Based on Genotypes

Symbol	Potential for Traits
S	Spatial perception (2 genes, each with 2 alleles) (3-4 S = excellent, 1-2 S = good, 0 S = fair)
I	Interpersonal skills (3 genes, each with 2 alleles) (4-6 I = excellent, 2-3 I = good, 0-1 I = fair)
M	Math ability (2 genes, each with 2 alleles) (3-4 M = high, 1-2 M = average, 0 M = fair)
V	Verbal skills (2 genes, each with 2 alleles) (3-4 V = excellent, 1-2 V = good, 0 V = fair)
C	Creativity (3 genes, each with 2 alleles) (4-6 C = fair, 2-3 C = average, 0-1 C = high)
B	Body build (2 genes, each with 2 alleles) (3-4 B = light, 1-2 B = average, 0 B = heavy)
P	Predisposition to heart disease (3 genes, each with 2 alleles) 4-6 P = low, 2-3 P = moderate, 0-1 P = high)
H	gene for unknown trait (1 gene with 2 alleles) (H/H = +, H/h = +, h/h = –)

netic profile is a part of your medical record. Who should and should not have access to this profile? Why?

Table 2.3 Examples of Careers

artist/musician
athlete
auto mechanic
baby sitter
bank teller
carpenter
chef
pilot/navigator
dancer
fashion designer
fire fighter
florist
health care provider
heavy-equipment operator
homemaker
investment counselor/stockbroker
plumber
counselor/social worker
scientist
teacher/principal

11. Suppose corporations based their recruitment and employment policies only on a genetic profile. What vital factor would they be ignoring?

12. In what ways do the traits in the genetic profiles illustrate the concept of human genetic variation?

13. How could you explain that even though brothers, sisters, and identical twins obviously are closely related, they often differ from each other in several ways?

14. Provide examples of human traits that do *not* result from multiple genes interacting with the environment.

15. Can one be sure that a certain genotype always will produce a certain phenotype? How does your answer cause concern for people who base their predictions solely on the genes that are present or absent?

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Activity 3
The Case of Nathaniel Wu
Copymaster

You probably have had some experience, either directly or indirectly, with discrimination and looking for a job. In this activity, you will analyze a dilemma that involves a young man's career and his attempt to secure a challenging job even though his genetic profile may indicate a potential health problem of which he previously was unaware. The dilemma involves ethics—the study of what is right and wrong and what is good or bad for individuals, institutions, and society. Although ethics as a field of study may sound obscure, you make ethical decisions all the time—for example, whether you should park in a parking space reserved for handicapped persons. Ethics establishes what should or should not be done on the basis of well-reasoned arguments. Well-reasoned arguments are sets of clearly stated reasons that together justify a conclusion. Well-reasoned ethical arguments can help us see the difference between justified and unjustified discrimination.

Part I Gathering and Evaluating Information

PROCEDURE

1. In Activity **2**, you determined the careers that were most suitable for certain genetic profiles. You also probably excluded certain profiles from certain careers. Why might it be justified to exclude a person who has a high predisposition to heart disease from a career as a pilot?

2. What about excluding a person who has blue eyes, or who is Hispanic or Jewish?

3. Discrimination means identifying differences, something we do all the time. In your notebook, write answers to the following questions:
   a) When is employment discrimination justified?
   b) When is employment discrimination unjustified?

4. Profile 1, which shows a potential for high math ability, high creativity, and excellent interpersonal skills, also has the genotype H/h. All other profiles have the genotype h/h. No trait was described for this gene. What might be its function?

5. Profile 1 belongs to a young man named Nathaniel Wu. Read ***The Case of*** *Nathaniel*

Wu. This case involves job discrimination based on the type of information the HGP may generate.

THE CASE OF NATHANIEL WU

Nathaniel Wu is a top-notch microbiologist. Now 30 years old, he has spent several years working in one of the best research laboratories in the world and has developed an excellent reputation as a creative researcher and hard worker. Following the birth of their son six months ago, Nathaniel and his 29-year-old wife decided it was time for Nathaniel to seek a job that could help them settle down and become financially secure. Thus, it was with great interest that Nathaniel read the following advertisement in a scientific journal:

> Intercontinental Pharmaceutical Corporation (IPC) of New Jersey is seeking highly qualified scientists to join a unique research team. IPC is prepared to invest up to $20 million in setting up and supporting a team of researchers to conduct creative research to find new treatments and cures for diseases ranging from AIDS to heart disease and the common cold. Because IPC will commit $20 million to this research effort, we will require those selected for this special research project to commit to a long-term employment contract. Interested applicants should send information to Dr. Anna Peters at IPC.

This was the type of job Nathaniel had always hoped for, and he applied immediately. Before long, IPC invited Nathaniel to come to its headquarters and interview for a position on this special research team.

Dr. Peters, the head of the research committee, led a series of interviews with Nathaniel and three other qualified applicants. Although the other three applicants also were well-qualified, they did not seem to share the same determination and drive as Nathaniel Wu. She listened carefully when Nathaniel presented his latest research findings to IPC scientists. They, too, were impressed with Nathaniel's knowledge, research skills, and potential for contributing to the special goals of the research team. Nathaniel sounded like the type of applicant who could have a long and productive career with IPC, and he seemed to be the sort of team player IPC was seeking.

Because Nathaniel was a top-quality research scientist, there was a high likelihood that his knowledge and research efforts might result in the type of discoveries for new drugs and treatments that were the goal of this special research project. Such discoveries and products could improve the quality of life for countless individuals and dramatically increase earnings for IPC. The investment by IPC of several million dollars to set up and support a laboratory for Nathaniel and to pay his salary while he worked seemed like a good one.

There was, however, one additional bit of information that Dr. Peters had before her as she considered her recommendation to the Employment Selection Committee. As part of the application process, Nathaniel had submitted a blood sample to determine his genetic profile, as had all other applicants. The profile showed that Nathaniel had the allele for Huntington disease. When asked about this, Nathaniel revealed that he knew nothing about his family history because he had been adopted as an infant. After thorough genetic counseling about the implications of this news, Nathaniel still wanted the job at IPC.

To have a clearer picture of the impact of this new information on her recommendation, Dr. Peters had requested information from the IPC medical director. The report included the following information:

> Huntington disease (HD) is an autosomal dominant genetic disorder with an incidence in North America of 1 in 20,000. It is extremely rare in Orientals. Individuals who have the allele for HD will, at some point, develop symptoms of the disease; the usual age of onset is between 35 and 45 years. The disorder is characterized by progressive degeneration of nerve cells in the central nervous system. The patient begins to have involuntary jerky or writhing movements of the arms and legs and facial grimacing. Changes in personality, including inappropriate laughter, crying, episodes of anger, memory loss, and bizarre, almost schizophrenic behavior may precede or follow the movement disorder; the clinical picture is highly variable. The disorder is fatal, with death commonly occurring when the patient is in his or her 50s, and the patient usually enters an almost vegetative state

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for the last few years of life. Although we cannot predict the precise age of onset of these symptoms, the fact that Nathaniel has lived to age 30 without any identifiable symptoms means that he has approximately a 60 percent likelihood of onset by age 40. Soon after the onset of symptoms, a person with Huntington disease most likely would be unable to perform safely or productively in a laboratory setting. Medical care for a patient with HD can be extremely costly, requiring long-term care in a hospital or other medical-care facility. Without testing Mrs. Wu, we can predict that their son has a 50 percent chance of having the allele for HD.

Dr. Peters faced a tough dilemma. Should she recommend that IPC hire Nathaniel Wu? On one hand, she knew that his skills as a scientist fit well with the special research project. He could help IPC develop new products and bring in a potentially large amount of revenue from his work in the laboratory. This would be to the advantage of IPC in the tough and competitive world of pharmaceutical manufacturing. She also knew that the goal of the special research team was to do long-term research, and no one could predict how long it would take to discover new drugs and treatments. She could not be certain how long Nathaniel would remain a productive scientist. IPC was investing large sums of money to support this special research project. Medical and other costs such as disability insurance, once Nathaniel developed symptoms, also weighed heavily as she considered whether to hire Nathaniel. Therefore, Dr. Peters decided to list the reasons for and against hiring Nathaniel for this special IPC research team and to take this information to the IPC Employment Selection Committee.

6. Write down one reason why IPC *should* hire Nathaniel Wu for the special research team and one reason why IPC *should not* hire him. Be prepared to discuss your reasons with other students.

7. Form small groups as directed by your teacher. In your small group, use Worksheet 3.1 to analyze *The Case of Nathaniel Wu*. You are to take the position of Intercontinental Pharmaceutical based on the information in the case. For the next 10 minutes, discuss the case and list as many reasons as possible that Dr. Peters might have for hiring or not hiring Mr. Wu to work on this special project at IPC. Ask yourselves "Is this fair? Should this be legal?" about each reason for or against hiring. Be sure to record all the ideas on your own worksheet.

8. Present your lists to the class and add any new ideas to your own worksheet.

QUESTIONS FOR DISCUSSION

1. It is certain Nathaniel will develop Huntington disease. What role does genetic variation play in this case?

2. What specific costs to IPC are at risk?

3. What potential benefits might Nathaniel Wu bring to the company?

4. In what ways is Nathaniel Wu qualified to do the assigned tasks? What unreasonable costs or risks to IPC would hiring Nathaniel Wu involve?

HOMEWORK ASSIGNMENT

Review both lists on your worksheet and put a star beside the three most powerful or convincing reasons in each column. Decide whether IPC should hire Nathaniel Wu. Explain your answer in a brief paragraph that includes what you think are the three strongest reasons in support of your position. Come to class tomorrow prepared to discuss your position and reasons.

Part II: Making Arguments and Arguing Ethical Issues

PROCEDURE

9. You will argue whether IPC should hire Nathaniel Wu. The discussion will take place in front of three of your classmates or other people your teacher selects. This three-person group will serve as the IPC Employment Selection Committee. Study the *Employment Hearing Questions* your teacher provides; the decision-makers will use these questions to decide whether IPC will hire Nathaniel.

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10. **Class:** Form small groups based on whether you answered *yes* or *no* to the question of hiring Nathaniel Wu. For the next 10 minutes, work together in your group, using each other's information and the *Employment Hearing Questions* to prepare an argument that will convince the decision makers of the group's position. Appoint a spokesperson who will make a brief opening statement (maximum 2 minutes) outlining your group's argument. At the hearing, the decision makers will allow each group to make an opening statement, and then they will conduct an open discussion.

    Decision makers: While the small groups are consulting, work together to compile your questions, review the instructions, and prepare to conduct the hearing as outlined in the *IPC Employment Hearing Guidelines.*

11. **Decision makers:** Conduct the hearing. When the hearing is over, retire to the hallway or elsewhere to decide the outcome. You have five minutes to prepare to explain the reasons for your decision.

    Class: While the decision makers are out, discuss the most powerful or convincing arguments of each side. Has anyone changed his or her mind? Why?

12. **Decision makers:** Present your decision to the class and explain how you arrived at that decision.

The next activity asks you to consider whether or not one's genetic profile (or a portion of one's genetic profile) should be public knowledge.

EXTENSION: THE AMERICANS WITH DISABILITIES ACT OF 1990

The Americans with Disabilities Act of 1990 makes it illegal to discriminate against qualified disabled workers. A disabled individual is defined as one who has (1) a physical or mental impairment substantially limiting at least one major life activity; (2) a record of disability (such as a cancer survivor); or (3) the perception of impairment (this likely will include asymptomatic persons who test positive for genetic disorders). The act also bars employers from questioning prospective workers about their past medical history, but permits them to test job applicants for genetic disorders that affect job performance. Employers can require medical exams after a job offer is made, but the results must be kept in a separate confidential medical file available only to the employee's supervisor, providers of emergency treatment, and the government.*

*From T. E. Morelli, "Protecting Worker Rights," *The Marker*, Spring 1992.

1. According to the wording of the Americans with Disabilities Act, at the present time neither Dr. Peters nor the Employment Selection Committee would have had access to Nathaniel's genetic profile. How would this restriction affect Dr. Peter's recommendation to the Employment Selection Committee? How would it affect the decision of the Employment Selection Committee?

2. Given the terms of the act, would *not* hiring Nathaniel Wu be an example of justified or unjustified discrimination?

3. Should the ADA be interpreted to provide protection for persons who may develop a genetic disorder but who do not yet show symptoms of the disorder?

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Worksheet 3.1 Analyzing The Case of Nathaniel Wu

Reasons for IPC to Hire Nathaniel Wu	Reasons for IPC Not to Hire Nathaniel Wu

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Activity 3

IPC Employment Hearing Guidelines

You are one of three corporate officers of IPC who together form the Employment Selection Committee. You will make the final decision whether to hire potential new employees. As officials of IPC, your first concern is to hire individuals who can best serve the interests of IPC.

You will conduct a hearing where your classmates will present arguments for and against hiring Nathaniel Wu. *Your most important responsibility during the hearing is to remain impartial while listening to the information and arguments. This will allow you to make the best decision for IPC.*

These instructions will help you conduct the hearing

- Read the case of Nathaniel Wu carefully, several times.
- Take notes during the arguments and presentations by your classmates.
- Come prepared with questions you would like answered to help you make the best decision for IPC.
- Avoid favoring one side or the other; call on each side during the hearing.
- The decision will be a majority vote based on answers to the following questions:
  1. Is Nathaniel Wu qualified to perform the tasks as outlined?
     a) Does he have the scientific knowledge and skills to do the research?
     b) Is he likely to sty with IPC to complete the goals of the research project?
  2. What are the potential benefits to IPC if
     a) we hire Nathaniel Wu?
     b) we don't hire Nathaniel Wu?
  3. What are the potential costs to IPC if
     a) We hire athaniel Wu?
     b) We don't hire Nathaniel Wu?
  4. What rights does IPC have as an employer in this situation?
  5. What rights does Nathaniel Wu have in this situation?
  6. What is the impact of the genetic variability of Huntington disease on our decision?
- After making your decision, you must present your decision and your reasons to your classmates.
- Conduct the hearing using this agenda:
  a) Welcome your classmates to the hearing.
  b) Explain that the purpose of this hearing is to decide whether it is in the best interests of IPC to hire Nathaniel Wu for the special research project.
  c) Listen to all opening statements from yes groups.
  d) Listen to all opening statements from no groups.
  e) Conduct an open discussion at which the groups can respond to your questions (10 minutes).
  f) Adjourn to make decision.
- Announce and explain the reasons for your decision as to whether IPC should hire Nathaniel wu.

Activity 3
Employment Hearing Questions

In preparing your arguments, consider the following list of questions. The IPC Employment Selection Committee will decide whether to hire Nathaniel Wu based on answers to these questions. You may want to address the most important of these questions in your opening statement.

1. Is Nathaniel Wu qualified to perform the tasks as outlined?
    a) Does he have the scientific knowledge and skills to do the research?
    b) Is he likely to stay with IPC to complete the goals of the esearch project?

2. What are the potential benefits to IPC if
    a) we hire Nathaniel Wu?
    b) we don't hire Nathaniel Wu?

3. What are the potential costs to IPC if
    a) we hire Nathaniel Wu?
    b) we don't hire Nathaniel Wu?

4. What rights does IPC have as an employer in this situation?

5. What rights does Nathaniel Wu have in this situation?

6. What is the impact of genetic variability of Huntington disease on our decision?

Left Wondering

By Gabrielle Hamilton

My parents decided when I was young that my brother Brian and I should know and understand about HD and our chances of inheriting it. My grandmother was in the late stages of the disease, and at that time our risk was just 25 percent.

When I was in school, my mother's older sister was diagnosed and she came to live with us. She was recently divorced from her husband of 15 years and had relocated to New York with her two daughters.

For the first time, I began to see HD through the eyes of a young adult. Now I noticed not just the constant and uncontrollable movements, but also the mental symptoms that can accompany the disease. Thinking of my cousins' new-found risk, I suddenly wanted to know even more about what could happen physically and emotionally to my mother, brother, or even me if we developed HD.

This was when I learned that if you inherit the gene, you inherit the disease. If you do not inherit the gene, you cannot pass it on to your children. From that point on, I quietly watched my mother for any signs of movements or jerks. Yet any I thought I had seen I would quickly deny.

Once, my mother and I were lying down at opposite ends of the sofa trying to take a nap, when I noticed that her foot would not stop tapping. When I asked her about it, she got very upset and yelled at me. After that, in an effort to prove that she wasn't burning off calories the way someone with HD often does, she went on a fattening diet and actually gained a few pounds. When I asked her about the disease again, she proudly announced that she couldn't possibly have it because she'd been able to gain weight. I believed her.

Four years later she was diagnosed with HD. She was told that she probably had the disease for at least four years. Unfortunately, the diagnosis did not come until after her relationships with family and friends were almost extinguished.

Two years after I'd gone away to college, my brother, unable to communicate with her, moved in with our father. My mother then focused her anger on me, and within a year of Brian's departure she cut me completely out of her life. This was in stark contrast to the 'best friends' relationship we'd enjoyed until then. The emotional toll on me was heavy.

Six months after graduating from Boston College, I decided to go through genetic testing for HD. I made this decision because I wanted to plan every part of my future, from a career to insurance policies, from relationships to children.

Taking the test meant making bimonthly trips to participate in a predictive testing research project at the Johns Hopkins Hospital in Baltimore, MD. Each visit lasted approximately three hours, half of it spent undergoing physical and cognitive testing to detect any early symptoms of the disease, the other half in counseling sessions to make sure that I knew what I was doing and to prepare me for all possible outcomes. Blood samples for DNA analysis were taken from my mother, her sister, my grandmother's sister, my father and myself. I was accompanied on the trips to Hopkins by either my fiance or my father and brother.

When I entered the testing program in April, I was told that it would take three to four months for the results to be known. As the counseling sessions came to an end, the tension was almost unbearable. Slowly, the days and weeks rolled by. Three months passed, four months. Additional samples were called for from other relatives. The agonizing wait continued.

Finally, on November 20, 1991, eight months after entering the program, the results came in: my tests was inconclusive. The pattern of inheritance of HD markers in my family was such that, without a DNA sample from my late maternal grandmother, my fate could not be determined. My risk of inheriting HD was still 50 percent.

During the counseling process at Johns Hopkins, I had been asked how I'd feel if the test proved inconclusive. I thought I'd feet relief, that I'd done all I could but that my knowing simply wasn't meant to be. Instead, I felt helpless. I had chosen to be tested for the sole purpose of ending the mystery and defining my own life. Yet I was still forced, like my parents and grandparents, to make the most important decisions of my life totally ignorant of what the future held for me.

Later, as I began to recover from my disappointment, I realized that even if I couldn't determine my long-term future, I could still set goals. I could be anything I wanted to be or do anything I wanted to do, but I had to start now.

So my first decision was to go back to school to study journalism. My mother's sister had been a journalist and then editor of the Weekend/Lifestyle section of a paper before she became ill. I also decided to continue to participate in the research project at Johns Hopkins. I travel down to Baltimore a few times a year for follow-up physical and cognitive testing. I'm hopeful that I will be among the first to be notified when researchers finally locate the gene.

I continue to lie awake at night thinking about HD. The worst part is the anticipation and the endless uncertainty. It's difficult not to contemplate the possibility that I may some day develop HD. I've always been a realist—I took the test because I wanted to be prepared for any eventuality. Yet I'm slowly learning to accept the fact that HD is a possibility, not a probability. And I'm not about to wait around for the uncertainty to end—I've got far too much to do.

Gabrielle Hamilton lives and works in New York City

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Activity 4
Public Policy: Genetics and Alcoholism
Copymaster

At some point, scientific discoveries and innovations move out of the laboratory and begin to have an impact on people. With the Human Genome Project, some of these effects are evident already. In the case of Nathaniel Wu, for example, a corporation had to decide what effect the results of genetic testing would have on its hiring practices. As the HGP generates more information about the genetic make-up of individuals, and as genetic testing becomes more widely available, society will face difficult questions about privacy, discrimination, family planning, and other controversial topics. Public policy, in the form of rules or regulations, is the formal mechanism by which society deals with such issues.

Public policy is a set of guidelines or rules developed by governmental agencies. When the government takes action, the result often is a law in the form of new legislation. Equally important, however, is the judicial system. By making decisions in cases that come before them, the courts have a great impact on public policy. Governmental agencies also can make public policy by *not* acting, and thus permitting individuals or institutions to act in any manner they choose with respect to the issue in question. Whether or not any law should be enacted usually depends, in part, on whether something should be done about a problem and whether the proposed law will be effective in correcting the problem.

In this activity, you will determine whether the government should pass a hypothetical law that restricts the purchase and use of alcohol by persons who have a genetic predisposition to alcoholism. This case study is hypothetical. There currently are no concrete data that show clear genetic predisposition to alcoholism.

PROCEDURE

1. Read the following information about alcoholism and its genetic basis.

   Alcoholism is a serious problem throughout the world. Studies indicate that 10-15 percent of Americans are alcoholics. Adverse consequences can arise from single bouts of drinking as well as from long-term alcohol abuse. At least 3 out of every 100 deaths in the United States can be attributed to alcohol-related causes. Motor vehicle crashes are the leading cause of injury deaths in the United States for individuals between the ages of 5 and 34. In 1990, 19,900 people in the United States died in traffic accidents that were alcohol-related.

Pregnant women who drink alcohol may give birth to children with fetal alcohol syndrome, in which the child has characteristic facial features and may be mentally retarded. Research also indicates that 20 to 36 percent of suicide victims have a history of alcohol abuse or were drinking shortly before their suicides.

Chronic, sustained consumption of alcohol has many damaging effects. Alcohol damages the liver and chronic users have an increased risk of liver disease (cirrhosis). In high concentrations, alcohol damages the lining of the intestinal tract and hampers the absorption of nutrients. The risk of cancer of the esophagus also increases with alcohol use. Many habitual alcohol abusers show signs of heart problems, and chronic alcohol consumption is associated with increased hypertension. Alcohol affects immune, endocrine, and reproductive functions. Heavy alcohol consumption also can cause neurological problems such as dementia, blackouts, seizures, hallucinations, and damage to the peripheral nervous system.

Alcohol abuse can affect not only the drinkers themselves, but also their spouses, children, friends, and employers, as well as strangers with whom they may have contact. In addition to traffic accidents, alcohol-involved injuries and deaths, serious medical consequences, and birth defects, alcohol abuse also is implicated in aggression, crime, marital discord, and loss of jobs.

Alcoholism, like many human behaviors, is very complex. No one yet is certain what causes a person to become alcoholic. Several lines of evidence suggest, however, that the tendency to some forms of alcoholism has a genetic component and runs in some families. A predisposition to alcoholism, however, is not the same as predetermination. As with many other conditions, it is common in some ethnic groups and rare in others. Psychological and social factors such as culture, peer-group influence, and experiences with the effects of alcohol influence an individual's drinking behavior. In light of the evidence from twin and adoption studies, however, it is possible that the HGP will identify "alcoholism susceptibility genes."

2. ***Suppose*** that scientists discover two alcoholism susceptibility genes (ASGs). Each gene has two possible alleles, a high-susceptibility allele and a low-susceptibility allele. Therefore, an individual may have 0, 1, 2, 3, or 4 high-susceptibility alleles. Almost everyone who has four high-susceptibility alleles would become an alcoholic if he or she were to consume alcohol on a regular basis. Individuals with no high-susceptibility alleles would have no genetic predisposition to become alcoholic. Intermediate genotypes would result in intermediate susceptibilities. Why might someone with four high-susceptibility alleles not become an alcoholic?

3. Further *suppose* that a legislator in your state government proposes the following law: "All applicants for a driver's license must be tested for susceptibility to alcoholism. Any individual who has 3 or 4 high-susceptibility alleles will have his or her license stamped with the words 'not eligible to purchase alcoholic beverages.' It will be unlawful for any such person to purchase alcoholic beverages. It will be unlawful to sell alcoholic beverages to anyone holding such a license. The unlawful purchase of alcohol will be punishable by the loss of the driver's license for two years. Selling alcohol to susceptible individuals will be punishable by a $10,000 fine. Giving alcohol to a person with such a license will result in a 60-day jail term."

   Use the worksheet to list the reasons that you would be for and against passing this law.

4. Form small groups as directed by your teacher. Use the Activity 4 worksheet you filled out to help you analyze whether or not the government should pass the alcohol law.

5. Write down your group answers to the discussion questions and report these, along with your group decision, to the rest of your class.

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QUESTIONS FOR DISCUSSION

1. What is the major question in this case study, and how does the proposed legislation attempt to address the question? Be concrete in your explanation.

2. What important information is missing about the alleles of the two alcohol-susceptibility genes?

3. What aspects concerning the causes of alcoholism are missing from the proposed legislation?

4. Contrast this proposed legislation with public policies concerning smoking.

5. What public policy questions might arise from the discovery of alcohol susceptibility genes?

6. What options or choices do we have in addressing the problem?

7. Who will be affected by each choice or option?

8. How will agencies that depend on tax money from the sale of alcohol be affected? What might be the effect of this legislation on liquor store owners, liquor manufacturers, hospital emergency rooms, insurance companies, alcohol rehabilitation centers, and other groups? Will the likely consequences benefit or harm them? How? How will their rights be respected or disrespected? Refer to your Activity 4 worksheet.

9. Given the results of questions 4 and 5, for each of the alternatives ask: Do we need to act now? Will the proposed solution work? Will the proposed solution be fair to all?

10. In 2 to 3 years, you will be eligible to vote for state officers. What would you like your representatives to do if such a law were proposed in your state?

11. How would you enforce the law if it were passed?

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Worksheet T4.1 Analyzing Public Policy: Genetics and Alcoholism

Reasons for Passing the Alcohol Law	Reasons for Not Passing the Alcohol Law

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MODERN GENETICS FOR ALL STUDENTS

Appendices

A. Timeline and Helpful Hints A3
B. Addresses of Frequently Used Suppliers A11
C. Some Presumably Simple Heritable Human Traits A13

APPENDIX A

Timeline and Helpful Hints

CHAPTER 1. DNA: THE HEREDITARY MOLECULE

SECTION A. What is DNA?

1. **An Introduction to DNA**
   10-15 minutes preparation time; 15 minutes class time*

2. **DNA in the News**
   10 minutes preparation time; 15 minutes class time*
   This is a good way to reinforce what students should already be aware of—that DNA, biotechnology, and genetics are such important topics in their lives that there is a wealth of information printed about them in the press. The purpose of this exercise is to begin the collection of information that can be used to help them throughout the unit, especially in the bioethics portion in Chapter 4.

SECTION B. What Does DNA Look Like?

1. **Spooling Purified DNA**
   60 minutes preparation time;15 minutes class time
   Although this is an easy exercise to prepare, it does take a little time. The preparation of the DNA may take several days to get the dehydrated DNA to go back into solution. Begin the prep work four or five days before you actually need the solution and all will work well. You will need a magnetic stirring plate to prepare this solution. Once the components of the solution are added to your mixing container, place the container on the magnetic stir plate and turn the plate on. The solution is finished when the liquid is completely clear and no clumps are visible.

2. **Extracting DNA from Calf Thymus**
   30 minutes preparation time; 30 minutes class time
   The hardest part of this activity is finding the calf thymus. Experience has shown that if you request the thymus from your grocery's meat department they can get it for you, but it will often take several days to a week to acquire. Once acquired, the thymus can be cut into chunks and frozen for years with no loss of quality for this lab experience. In an emergency, chicken livers can be used, but the amount of DNA should be less.

*photocopy time

SECTION C. What is the Structure of DNA?

1. **The Puzzle of DNA Structure and Replication**
   *120 minutes preparation time (one time only)**; 20 minutes class time*
   Have the puzzle pieces copied on colored paper and laminated. Cut them out and store them in envelopes or plastic bags. The laminated pieces can be kept and reused for years. Remember to have extra sheets of the puzzle pieces made and laminated, so that when pieces are lost or damaged, you will have replacements for them. Cutting the pieces can be done either by you or the students. Experience shows that teachers are more careful in this than most students.

2. **"The Spiral Staircase" from *The Cartoon Guide to Genetics***
   No preparation time; 10 minutes class time

3. **What is a Model? And What is it Good For?**
   No preparation time; 10 minutes class time

4. **Building a Three-Dimensional DNA Model**
   No preparation time; 20 minutes class time

5. **DNA Model Questions**
   10 minutes preparation time; 20 minutes class time*

6. **DNA Word Search**
   10 minutes preparation time; 20 minutes class time*

SECTION D. What Does DNA Do?

1. **DNA Codes for Proteins**
   No preparation time; 10 minutes class time

2. **"How DNA Codes for Proteins" from *The Cartoon Guide for Genetics***
   No preparation time; 10 minutes class time

3. **The Gene Expression Dance**
   *30 minutes preparation time (one time only)**; 15 minutes class time*
   Since this is a whole class role-play, you really need only one set of puzzle pieces. Have the puzzle pieces copied on colored paper and laminated. Some of the puzzle pieces will be two-sided, and care should be used to insure that the proper front and back sheets are laminated together. Cut them out and store them in envelopes or plastic bags. The laminated pieces can be kept and reused for years. Remember to have extra sheets of the puzzle pieces made and laminated, so that when pieces are lost or damaged, you will have replacements for them. Cutting the pieces can be done either by you or the students. Experience shows that teachers are more careful in this than most students.

*photocopy time   **cutting out paper models; could be done in class

4. **Paper Proteins: Models for Simulating Gene Expression**
   *120 minutes preparation time (one time only)**; 30 minutes class time*
   Have the puzzle pieces copied on colored paper and laminated. Cut them out and store them in envelopes or plastic bags. The laminated pieces can be kept and reused for years. Remember to have extra sheets of the puzzle pieces made and laminated, so that when pieces are lost or damaged, you will have replacements for them. Cutting the pieces can be done either by you or the students. Experience shows that teachers are more careful in this than most students.

5. **Using the Genetic Code to Translate an mRNA**
   10 minutes preparation time; 15 minutes class time*

SECTION E. How Does DNA Determine a Trait?

1. **An Introduction to the Connection Between Genes and Visible Traits**
2. **Shine On!**
   a. How to Read a Micropipettor
   b. Practicing Microbiological Techniques
   Bacteria for this section can be streaked onto plates several days to a week before needed and then kept in the refrigerator. Strive to get plates with single colonies so that your students can pick a single colony to start their liquid cultures.
   c. Engineering Glow-in-the Dark Bacteria
   It is essential that the *E. coli* cultures used in this activity be freshly grown and no more than 24 hours old. The day before they are needed, you will need to streak enough plates so that each lab group will have a culture of bacteria to work with. It is best to have plates with individual colonies growing on them, so that the point can be made that all of the cells from a single colony come from a single bacterium. If your plates have few or no colonies, but a lawn or large masses of bacteria on them, the protocol will still work well. NOTE OF CAUTION: After the completion of the transformation protocol DO NOT incubate these plates at 37°C. Use a 30°C incubator or room temperature. The inserted glow-in-the-dark genes will not express themselves properly under the warmer conditions.

CHAPTER 2. PASSING TRAITS FROM ONE GENERATION TO THE NEXT

SECTION A. What is Inheritance?

1. **An Introduction to Inheritance**
   10 minutes preparation time; 15 minutes class time*

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*photocopy time **cutting out paper models; could be done in class

SECTION B. How Does a New Generation Get Started?

1. **Model Systems for Studying Heredity and Development**
2. **Starting a New Generation: Sea Urchin Fertilization**
   If you order the urchins from Carolina Biological, **you must order them at least three weeks before you need them.** They will be harvested on the Monday of the third week and shipped the same day. The urchins will arrive by Wednesday or Thursday at the latest. You will need to make your receiving department aware that you are expecting the urchins so that you will be notified about their arrival and can deal with the urchins quickly. To accomplish this, you will also need to have the aquaria prepared ahead of time. Experience has shown that setting up the aquaria on Monday or Tuesday gives time for the water temperature to stabilize and for you to get the salts in balance before adding the urchins. **Note:** when cleaning the aquaria after use, do not use soaps or detergents—the residue can harm the urchins you get next year.

3. ***The Miracle of Life***
   No preparation time; 60 minutes class time

SECTION C. If All the Kids Have Mom and Dad's Genes, Why Don't They All Look Alike?

1. **Really Relating to Reebops**
   20 minutes preparation time; 45 minutes class time
   For this laboratory to work well, the marshmallows need to be stale. (When they are too fresh, they are mashed by the addition of the various parts–legs, eyes, extra body segments, etc.) Simply open the bag of marshmallows, spread them out on a tray and keep them in a cool dry place for about a week. The marshmallows will be ready for use when they do not compress too much when squeezed between the thumb and forefinger.

2. **Determining Genetic Probabilities with a Punnett Square**
   5-10 minutes preparation time; 20 minutes class time*

3. **Exploring Human Traits: Create-a-Baby**
   10 minutes preparation time; 30 minutes class time*
   Bring coins for the students to use.

4. **Using a More Complicated Punnett Square**
   10 minutes preparation time*; 20 minutes class time

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*photocopy time

SECTION D. How are Genetic Experiments Actually Performed?

1. **A Colorful Experiment in Yeast Genetics**
   120 minutes preparation time; 30 minutes class time each for two days
   This laboratory exercise is wonderful for showing the expression of dominant and recessive alleles as well as a very graphic review of Punnett squares. Producing YEAD culture plates takes no longer than preparing or pouring regular plates. However, you must allow them to dry completely so that condensation will not fall on the growing yeast and flow from one growth area to another, causing cross contamination of cultures on the plate. Make the plates at least one full week before they are needed. Check through the plates daily, removing and bagging those whose lids are free of condensation. The condensation-free plates are ready to use and can be kept in the refrigerator for four to six weeks. Some of the plates that you have poured may have condensation on the lids for up to two weeks. When the students inoculate the YEAD plates with yeast, make sure they are very careful of contamination by airborne spores. The yeast will grow best at 30°C, but can be grown at room temperature. The Reds will be at their peak of color in three days of 30°C incubation or five to seven days at room temperature. The extra time is worth the effort for the results obtained.

2. **Experimenting with Wisconsin Fast Plants**
   180 minutes preparation (one time only); 45 minutes class time for the first day, 10 minutes for other days
   This exercise is good for teaching constants and variables in experimental design as well as reinforcing the skills of graphing and data collection. The most difficult part of this exercise is the preparation of the plant lighthouses and the film-can growth systems. Once the lighthouses and growth systems are constructed, they can be used year after year with good results. There is no quick way to make the plant lighthouses or the film-can growth systems, so follow the directions and take your time and you will get great results.

SECTION E. How are Genetic Results Evaluated Statistically?

1. **Introduction to Using Statistics to Evaluate Genetic Explanations**
   10 minutes preparation time; 15 minutes class time*

2. **Too Many White Kittens? Using Chi Square (χ^2) to Find Out**
   10 minutes preparation time; 20 minutes class time*

3. **How to Perform a Chi-Square Test on Any Data Set**
   10 minutes preparation time*; 20 minutes class time

*photocopy time

CHAPTER 3. HOW GENES AND THE ENVIRONMENT INFLUENCE OUR HEALTH

SECTION A. How Stable and How Powerful Is DNA?

1. **DNA Paradoxes**
   10 minutes preparation time; 10 minutes class time*

SECTION B. How Do Heritable Changes in Genes Occur?

1. **Inducing Mutations with UV Light**
   60 minutes preparation time; 45 minutes class time for day one, 20 minutes class time for day two

SECTION C. Is it Nature, or Is It Nurture?

1. **Albino Plants: A Model for Gene-Environment Interaction**
   45 minutes preparation time; 20 minutes class time for each of two days
   Here you will again prepare agar plates. This time you will not need to sterilize the agar because there are no nutrients present that bacteria or fungi can use for food. The difficult part of this preparation comes when the charcoal powder is added. The trick is to get the agar completely dissolved, add the charcoal powder and then get everything thoroughly mixed and poured before the whole mixture cools too much. The black agar that is produced is an excellent background for showing the phenotypes of the sprouting tobacco seeds. This exercise is a great one, not only for the 3:1 ratio portrayed but also for showing how the environment affects the expression of the pigment genes in the tobacco.

2. **Heart Disease: A Personal Gene-Environment Interaction**
   10 minutes preparation time; 30 minutes class time*

SECTION D. What Are Some of the Features of "Simple" Genetic Diseases?

1. **Some "Simple" Heritable Defects**
   10 minutes preparation time; 20 minutes class time*

2. **Phenylketonuria (PKU) Illustrates the Complexities of Some "Simple" Genetic Diseases**
   10 minutes preparation time; 20 minutes class time*

3. **The Special Inheritance Patterns of Sex-Linked Mutations**
   10 minutes preparation time; 20 minutes class time*

4. **Investigating Human Genetic Diseases**
   10 minutes preparation time; 20 minutes class time*

*photocopy time

SECTION E. How Does a Genetic Counselor Detect Mutant Genes?

1. **Detecting the Duchenne Muscular Dystrophy (DMD) Mutation**
   See note for preparation time; 45 minutes class time
   This exercise is a great simulation of how scientists detect specific alleles in someone suspected of having them. If the agarose gels for the electrophoresis simulation are precast and your students work the entire period, you can get this investigation finished in one normal class period. Either you or your students can cast the gels the day before and then store them overnight in a plastic bag with water for the next day's class. Casting the gels will take 20-30 minutes for your students. However, you will have to cast as many gels as you have gel boxes for, remove them to plastic bags and then repeat for each of your classes. This process can take several hours. Making the dyes may take up to an hour but, if made in large quantities, the dyes for this investigation can be stored for years. While the dyes are running on the gels, there is a block of time that could be used in class to do other work.

SECTION F. How Can I Become a Genetic Counselor?

10 minutes preparation time; 20 minutes class time*

CHAPTER 4. CONTROLLING OUR GENETIC FUTURES

SECTION A. Biotechnology: Panacea or Pandora's Box?

1. **Video–Promise & Perils of Biotechnology: Genetic Testing**
   10 minutes preparation time; 45 minutes class time*
   The pre-video worksheet questions take five minutes. Twenty five minutes are required to show the video and fifteen minutes are needed for the post-video worksheet questions and discussion.

2. **Worksheet for Promise & Perils of Biotechnology: Genetic Testing**
   10 minutes preparation time; 20 minutes class time*
   See section A-1 above.

SECTION B. Resolving Genetic Testing Issues: An Introduction to Group Decision Making

1. **A Value-Based Approach to Group Decision-Making**
   10 minutes preparation time; 35 minutes class time*
   The reading for this can be done in class, and will take from five to ten minutes. It will then take another five to ten minutes to complete the individual work. When the individual sheets are completed, fifteen to twenty more minutes will be needed to come to a group decision on "The Class List" worksheet.

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*photocopy time

2. **New Genetic Tests Lead to Difficult New Questions**
   10 minutes preparation time; see below for class time*
   Experience has shown that this section can take anywhere from a few minutes to a few days to complete. Student are interested in many of the ideas incorporated in this section. You might think of having a group discussion about each item or allow the students to pick three or four items to cover. Encourage the group to decide which way to go based on the criteria expressed in "The Class List" worksheet from the previous section.

SECTION C. Genetic Testing: Two Case Studies

1. **Roger Patton's Dilemma**
   10 minutes preparation time; 30-40 minutes class time*
   Class time will vary based on whether you assign this reading for homework or have it done in class. It should take you from five to ten minutes to give and overview. It should take another five to ten minutes for the students to fill out the work sheet. After completion of the work sheet, there will need to be time allotted to discuss student ideas from their work sheets.

2. **Carol and George Face a Tough Decision**
   10 minutes preparation time; 30-40 minutes class time*
   Class time will vary based on whether you assign this reading for homework or have it done in class. It should take you from five to ten minutes to give and overview. It should take another five to ten minutes for the students to fill out the work sheet. After completion of the work sheet, there will need to be time allotted to discuss student ideas from their work sheets.

SECTION D. DNA in the News

Depending on how you have chosen to use the news articles collected, this section could take several days to complete. The key element of this section is to let students know early in this unit what you expect them to do with the articles. You may choose to have students prepare and present a poster of articles collected on specific genetics related topics; you may choose to have students use the collected articles as a basis for a term paper. You may want your students to use the information as background for a panel discussion of genetics issues. Some teachers have even had groups of students prepare arguments that could be used in a debate. Or you may choose all or none of these ideas.

*photocopy time

APPENDIX B

Some Frequently Used Suppliers

Carolina Biological Supply Company
2700 York Road
Burlington, NC 271215-3398
Tel: (800) 334-5551
Fax: (800) 222-7112
www.carolina.com

Fisher Scientific
(Many addresses throughout USA)
Tel: (800) 766-7000
Fax: (800) 926-1166
www.fishersci.com

Fisher Scientific Education
485 South Frontage Road
Burr Ridge, IL 60521
Tel: (800) 955-1177
Fax: (800) 955-0740
www.fisheredu.com

Nasco Science
901 Janesville Ave.
Fort Atkinson, WI 53538-0901
Tel: (800) 558-9595
Fax: (920) 563-8296
www.eNASCO.com

Sigma
P.O. Box 14508
St. Louis, MO 63178
Tel: (800) 325-3010
Fax: (800) 325-5052
www.sigma-aldrich.com

Ward's Natural Science Establishment
P.O. Box 92912
Rochester, NY 14692-9012
Tel: (800) 962-2660
Fax: (800) 635-8439
www.wardsci.com

APPENDIX C

Some Presumably Simple Heritable Human Traits

MANY ITEMS LISTED in this table are considered tentative, because the heritable basis for some of these traits has not been fully resolved. Others are somewhat misleading, because (as noted for some entries) certain traits have a different genetic basis in occasional individuals than they do in most other affected persons.

A. HAIR

DOMINANT	RECESSIVE
Curly	Straight
Dark	Light blond
Light red	Intensely red
Red tint to blond or brown	No red pigment at all
White forelock	No white forelock
Prematurely gray (white before age 30)	No premature graying
Widow's peak	Straight hairline
Clockwise whorl	Counterclockwise whorl
Male-pattern baldness in men	Male-pattern baldness in women

Despite the fact that male pattern baldness is much more common in men than women, is not a sex-linked condition. It is an example of an autosomal condition that is sex-limited, which means that symptoms are strongly influenced by the sex hormones. Thus, male-pattern baldness is a dominant trait in men, and also in women who are taking testosterone for medical purposes, or in women who have a deficiency of female sex hormones for some reason. But it is a recessive trait in women with normal hormone levels.

Excessive facial hair in women	No excessive facial hair in women
Hair on middle segments of fingers	Middle segments of fingers hairless

B. EYES

DOMINANT	RECESSIVE
Brown	Grey

Contrary to earlier opinion, blue eye color is not a simple autosomal recessive trait; it is a multigenic trait.

One eye dominant	Two eyes used equally

Only about 5 percent of the population use both eyes equally; about 65 percent favor their right eye, and about 30 percent favor their left eye.

Normal vision	Severe nearsightedness in childhood

Autosomal-dominant and sex-linked forms of nearsightedness (myopia) are also known.

Astigmatism	Normal vision
Red-green color blindness in men	Red-green color blindness in women (sex-linked)
Normal blue-yellow color vision	Blue-yellow color blindness

Blue-yellow color blindness is much less common than red-green color blindness, and although it is usually transmitted as an autosomal recessive condition, autosomal dominant and sex-linked forms are also known.

Extremely long eyelashes	Normal eyelashes
Sudden bright light causes sneezing	No sneezing triggered by light

C. EARS

Free earlobes	Attached or missing earlobes
Rim of ear rolled	Rim of ear flat
Pointed on top	Round on top
Ears can be wiggled without touching them	Ears cannot be wiggled this way
Tone deafness	Normal tone discrimination
Normal hearing	Born deaf

Only about 30 percent of the cases of childhood deafness can be clearly attributed to genetic causes, about 40 percent are the result of known non-genetic causes (such as ear infections), and about 30 percent have unknown causes. A number of different autosomal recessive mutations can result in deafness, but an autosomal dominant form is also known.

D. FINGERS AND TOES

DOMINANT	RECESSIVE
Extra fingers and/or toes	Normal number of digits
Fingers numb and cold from poor blood flow	Normal blood flow in fingers
Thumb permanently flexed	Thumb can be flexed or straightened
Thumbs and big toes stubby and broad-ended	Normal thumbs and toes
All fingers and toes short and stiff	Normal fingers and toes
Second toe longer than big toe	Big toe longer than second toe

E. TONGUE

Can be rolled up from the sides	Cannot be rolled up from the sides
Tip cannot be folded backward	Tip can be folded backward
Taster (thinks PTC tastes bitter)	Non-taster (thinks PTC has no taste)

PTC = phenylthiocarbamide. For reasons that are not at all understood, tasters have a higher incidence of thyroid cancer than non-tasters do, whereas non-tasters have hyperactive thyroid glands more frequently than tasters do.

F. TEETH

Present at birth	Emerge weeks later
Extra teeth	Normal number
Develop normally	Baby teeth OK; no permanent teeth develop
Peg-shaped	Normally shaped
Protuberant upper front teeth	Normal upper front teeth
Many fail to emerge from gum (sex-linked)	Emerge normally

G. NOSE

DOMINANT	RECESSIVE
Normal	Turned up at tip
Wide nostrils	Normal nostrils
No polyps	Nasal polyps
Cannot smell skunk	Can smell skunk
Cannot smell musk	Can smell musk
Can smell chemical from asparagus in urine	Cannot smell this compound
Cannot smell cyanide (sex-linked)	Can smell cyanide
Generally deficient sense of smell	Normal sense of smell

A sex-linked version of the deficient sense of smell also exists.

H. SKIN

Freckles	No freckles
Dimple on cheek	No cheek dimples
Dimple in middle of chin	No chin dimple
Dimple on back of shoulder	No shoulder dimples
Stretch marks around lower back	No such marks
Excessive, painful calluses on hands and feet	Normal calluses
Multiple pigmented moles	Few or no moles
Chilblains	No chilblains

Exposure to cold causes red or blue discoloration and swelling of skin, accompanied by intense itching and/or burning sensations. Most common in young women.

Hypersensitive to cold	Normal cold sensitivity of skin

Differs from the above. Exposure to cold causes skin wheals, pain and swelling of joints, chills and fever.

Spicy foods cause prolific sweating	No such effect
Varicose veins	No varicose veins

I. NIPPLES

DOMINANT	RECESSIVE
Inverted	Normal (protruding)

Inverted nipples are more common in women than men; may be sex-limited.

Extra (sometimes with extra breasts)	Two

Extra nipples are more common in women who are identical twins.

J. URINE

Normal color after eating beets	Red after eating beets
Foul smelling after eating asparagus	Normal smelling after eating asparagus

E.2.c

Name ______________________________

Date ________________ Hour ________

LABORATORY WRITE UP

1 Purpose: Describe in your own words the reason for performing this experiment.

2. Background Information: Give information about the idea of adding genes to bacteria in order to change their traits.

3. Hypothesis: State the expected outcome of the experiment.

4. Independent Variable: State the independent variable.

5. Dependent Variable: State the dependent variable.

6. Controls: List the controls.

7. Procedure: Briefly describe in your own words the steps you took to perform the experiment.

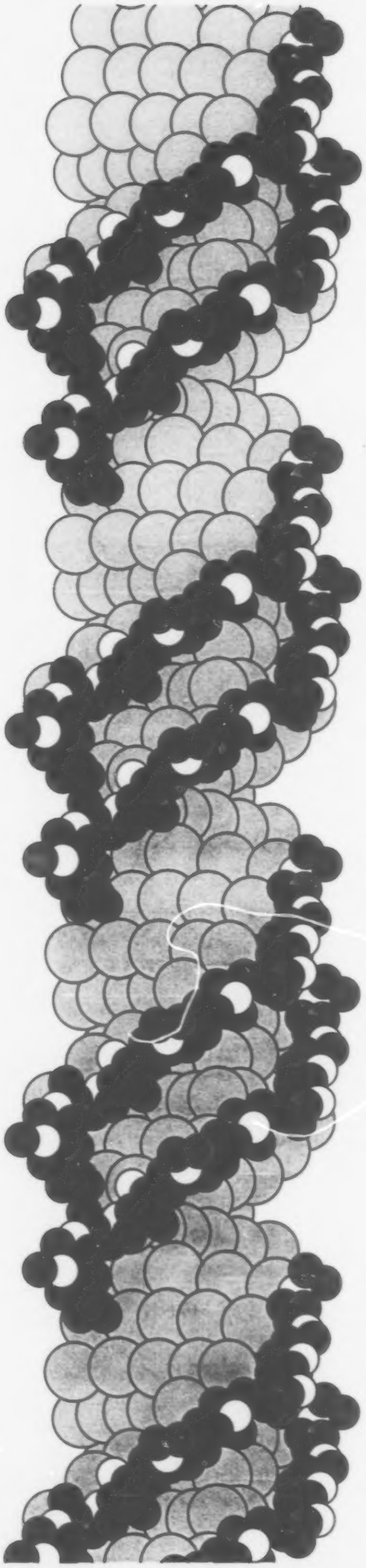
E.2.c

Name ______________________

Date ______________ Hour __________

8. Data/Observations: Organize your data and observations into a neat, meaningful chart.

9. Conclusions and Recommendations for Future Experiments: Tell what the data mean. Were the results what you expected? Was your hypothesis on target? In addition, write a paragraph telling your ideas for future experiments (how to improve, what other things to try, any mistakes to correct, etc.).



CHAPTER 1

DNA: The Hereditary Molecule

CHAPTER 1

DNA: The Hereditary Molecule

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CHAPTER 1

DNA: The Hereditary Molecule

SECTION A

What is DNA?

A.1

An Introduction to DNA

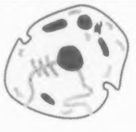
DID YOU REALIZE that you came with a complete set of instructions for assembly? They weren't printed on paper like the ones that come with a new bicycle; they were written in a special code, in a substance called **deoxyribonucleic acid**, or **DNA**.

The instructions in your DNA are called your genes. Your genes told all of your cells how to grow, when to divide, and how to move. Working together with each other and many external factors (such as the amounts and kinds of foods that you have eaten), your many thousands of genes have determined all of your traits, such as the shape of your facial features, how tall you are, how much you weigh, and how healthy you are.

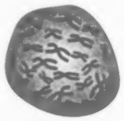
It is important to realize, however, that although certain bits of our DNA code for the special traits that makes each of us different, most of our DNA codes for the basic features of life that each of us shares with every other human being, as well as with every other living thing.

WHERE DID YOUR DNA INSTRUCTIONS COME FROM?

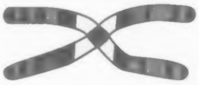
Half of your DNA came in a sperm cell from your father and half came in an egg cell produced by your mother. When those two cells fused to form a new cell — a fertilized egg — this cell used the instructions in its DNA to grow and divide many times and become you.



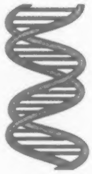
THE HUMAN CELL
Each of the 100 trillion cells in the human body (except red blood cells) contains the entire human genome — all the genetic information necessary to build a human being. This information is encoded in six billion base pairs, subunits of DNA. (Egg and sperm cells each have half this amount of DNA.)



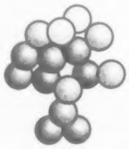
THE CELL NUCLEUS
Inside the cell nucleus, six feet of DNA are packaged into 23 pairs of chromosomes (one chromosome in each pair coming from each parent).



A CHROMOSOME
Each of the 46 human chromosomes contains the DNA for thousands of individual genes, the units of heredity.



A GENE
Each gene is a segment of double-stranded DNA that holds the recipe for making a specific molecule, usually a protein. These recipes are spelled out in varying sequences of the four chemical bases in DNA: adenine (A), thymine (T), guanine (G) and cytosine (C). The bases for interlocking pairs can fit together in only one way: A pairs with T; G pairs with C.



A PROTEIN
Proteins, which are made up of amino acids, are the body's workhorses — essential components of all organs and chemical activities. The function of each protein depends on the sequence of amino acids that it contains, which in turn depends on the sequence of A's, G's, C's and T's in one of the 50,000 to 100,000 genes in the cell nucleus.

Because brothers and sisters each receive half of their DNA from their father and half from their mother, they often resemble one another. But because each of them gets a slightly different set of DNA instructions from each parent, no two children look exactly alike — except in the rare case of identical twins.

JUST HOW DOES YOUR DNA DETERMINE YOUR TRAITS?

Your DNA contains many different sections called **genes** that contain coded instructions for making different kinds of proteins. Each kind of protein has a special effect on any cell that contains it. For example, certain genes that you inherited from your mother and your father determined what kinds of proteins were made in the cells forming your hair, and thus determined what color your hair would be.

All of us are distinguishable individuals, because all of us have our own special combinations of small DNA regions that influence various aspects of our physical appearance. But as mentioned above, these regions of our DNA that distinguish us from other people are the exceptions rather than the rule. More than 99.9% of our DNA is identical to that of every other human being.

The laboratory experiences in this chapter will help you to understand more about this material called DNA and how it controls both the shared and the specialized features of yourself and every other organism on our planet.

A.2

DNA in the News

LESSON OVERVIEW

DNA GETS MENTIONED in newspaper and magazine articles with increasing frequency these days. It certainly gets more publicity than any other type of molecule. DNA evidence regularly plays a central role in highly publicized court cases involving assault, murder, or rape. There are also an increasing number of cases reported in which a person who was convicted of a violent crime many years ago has been set free due to DNA evidence. This can happen when samples collected at the time of the crime are analyzed with modern methods, and the DNA in them establishes the convict's innocence. In news of a very different sort, newspapers regularly announce major breakthroughs in the identification of changes in DNA that are thought to be the cause of various serious human diseases. Although DNA research holds much promise for families afflicted with heritable diseases, this research is not without controversy. Many articles can be found that discuss the intense controversies that are raised by the patenting of human genes, the development of new kinds of genetically engineered plants or animals, or the issue of labeling genetically modified foods.

The purpose of this exercise is to establish a collection of such articles in your classroom that will serve as the basis for classroom discussions of various aspects of modern genetics. Starting today, you should search any newspapers or magazines that you have access to either at home, in the library or elsewhere for articles about DNA or any other topic in genetics. If you have access to the Internet, you may also wish to search it. Clip or make a copy of any such article that you find and bring it to school to share with your teacher and your classmates.

You should write a brief summary to accompany each article that you collect and then give the article and your summary to your teacher. Your summary should cover the following:

- title of the article,
- author(s) (if identified),
- name of the newspaper, magazine, or other source in which the article appeared,
- date of publication,
- volume, issue, and page number(s),
- brief summary of the main points of the article (Who did what, where, when, why, and how?).

Your teacher will give you additional instructions about how such articles will be displayed, archived, and discussed by the class.

Note: Although you should get started on this project right away, you should continue searching for and collecting relevant articles as long as you and your classmates continue to study genetics.

CHAPTER 1

DNA: The Hereditary Molecule

SECTION B

What Does DNA Look Like?

B.1

Spooling Purified DNA

INTRODUCTION

DNA IS THE MATERIAL that contains all of the instructions that are required for building your cells and keeping them alive. Each of the 46 chromosomes in one of your cells contains one DNA molecule that is an inch or two long, though it is far too slender to be seen with any but the most powerful electron microscopes. If we could enlarge one of these DNA molecules enough so that we could see it — let's say so that it was about the same diameter as one of the hairs on your head — we would find that it was several miles long!

In this exercise, we will take advantage of this long, thin, "threadlike" shape of DNA molecules to "spool" them, which is to say, we will wind them up on a wooden stick like a piece of thread. As you probably know, cotton fibers (which are individually a couple of inches long and quite thin) can be combined into one long, continuous thread, because they tend to stick to one another and line up side by side. The same thing can happen when DNA molecules come out of solution — if we pull on them from one end. We will do this by slowly twisting a stick in the region where DNA is beginning to precipitate. Each DNA molecule that is initially caught and wound around the stick will catch and pull on several other molecules, each of which will then catch and pull on others. Thus, if we work carefully, we can wind all of the DNA molecules in a test tube into one long, continuous thread. Although DNA molecules are so thin that you couldn't possibly see one of them with your naked eyes, if you wind up many such molecules together (as you will in this exercise), the DNA becomes visible, and its properties can be studied.

MATERIALS

For each group of four students:
- 1 test tube of DNA
- 1 test tube of alcohol
- 1 wooden stick

PROCEDURE

1. Your teacher will give you a test tube containing a solution of DNA (which was isolated from salmon sperm by a procedure rather similar to the one described in the next exercise), another test tube containing alcohol, and a wooden stick. (Fig. 1) Record your observations of the liquids in the two tubes on your Observations sheet. Can you tell which one is the DNA? How?

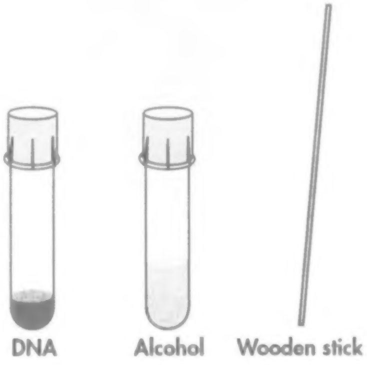


Figure 1

2. Uncap the tubes and hold the tube that contains the DNA at an angle (Fig. 2).

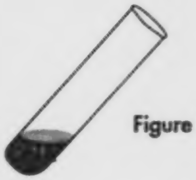


Figure 2

3. Carefully transfer the alcohol from its tube into the DNA tube (Fig. 3). Pour very slowly, so that the alcohol does not mix with the DNA solution and stir it up. What does the alcohol do? Record your observations.

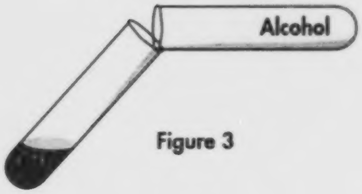


Figure 3

4. Gently insert the wooden stick through the alcohol layer to the interface where the two liquids meet (Fig. 4). Twirl the stick gently.

5. Now slowly lift the stick from the tube and observe the material clinging to it (Fig. 5). How long a fiber can you pull from the tube?

6. Put the stick back in the tube and gently twirl it in the vicinity of the interface again. Can you get more DNA attached to the stick?

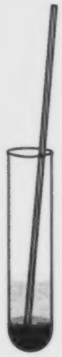


Figure 4

7. When you have finished with the stick, cap the tube and shake it several times (Fig. 6). Do you see more DNA in the tube now?

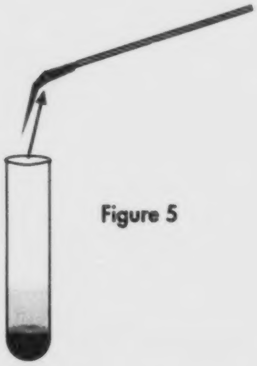


Figure 5

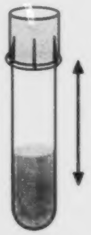


Figure 6

B.1

Name ____________________

Date ____________ Hour ________

OBSERVATIONS

1. Describe the appearance of the liquids in the two tubes.

2. Can you tell which of the tubes contains DNA? How?

3. Describe what happened when you first twirled the stick in or near the DNA-alcohol interface.

4. When you lifted the stick out of the tube and a fiber of DNA followed, did you think that this was a single molecule of DNA? Why?

5. How would you describe the appearance of DNA to someone who has never seen it?

6. What do you think it is about the biology of salmon and sperm cells that makes it easy to isolate a large quantity of DNA from salmon sperm?

B.2

Extracting DNA From Calf Thymus

INTRODUCTION

AS WE HAVE LEARNED, DNA is one of the most important substances in the world, because it carries the coded instructions for making all sorts of other molecules that are required for life. How does DNA do this? We'll get to that part later. For now, what we want to do is to see how purified DNA, such as we examined in exercise B.1, is isolated from cells and tissues.

MATERIALS

Materials for this exercise will be found at four different distribution stations, as follows.

Station 1:
"Thymus soup"

Station 2:
Woolite or dishwashing detergent and dropping pipettes

Station 3:
Adolph's Meat Tenderizer and small scoops or spatulas

Station 4:
Alcohol, wooden sticks and paper towels

PROCEDURE

1. At Station 1 pick up a tube of "thymus soup" that your teacher has prepared by blending a piece of calf thymus in water. Observe the thymus soup and record what you see on your OBSERVATIONS sheet.

2. At Station 2 use a dropping pipette to add a small amount of detergent (Woolite or dishwashing detergent) to the thymus soup (Fig. 1). Do not shake the tube vigorously or it will foam too much. Just swirl the tube gently to mix the two fluids. Record your observations.

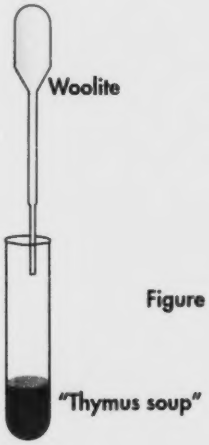


Figure 1

3. At Station 3 use the small scoop to add a small amount of Adolph's Meat Tenderizer to the tube (Fig. 2). Again, swirl the tube gently; do not shake. The tenderizer will not dissolve completely, but that's fine. Record your observations.

4. Wait five minutes for the meat tenderizer to work. Use this time to finish answering questions 1-5 on your OBSERVATIONS sheet.

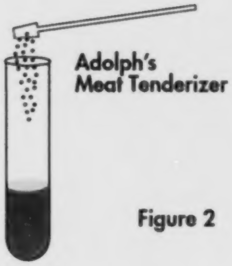
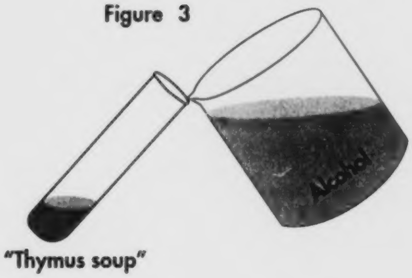


Figure 2

5. At Station 4 tip your tube slightly and then pour alcohol from the beaker so that it runs gently down the side of the tube and forms a layer 1-2 cm deep over the thymus soup (Fig. 3). Try to avoid stirring up the thymus soup as you add the alcohol.

Figure 3



6. Pick up a wooden stick and a paper towel at Station 4 and return to your work area.

7. Now things get more interesting! Insert the stick into the tube as far as interface where the thymus soup and alcohol meet and twirl the stick once or twice gently, just as you did in the previous exercise (Fig. 4). What happens?

8. Slowly pull the stick out of the fluid (Fig. 5). Now what is happening?.

9. Return the stick to the liquid and wind up as much DNA on the stick as you can. If you would like to save your DNA for later, you can wipe it off on the paper towel and take it with you when you leave the classroom.

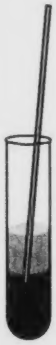


Figure 4

The method you have just used to isolate DNA from calf thymus resembles the methods that law-enforcement agencies use to isolate DNA from samples found at crime scenes.

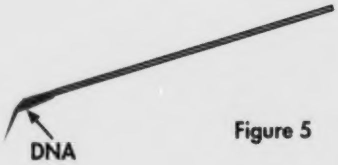


Figure 5

B.2

Name ____________________

Date ____________ Hour ________

OBSERVATIONS

Describe what you see as you perform the steps to isolate DNA.

1. What does the "thymus soup" look like?
2. How does its appearance change as you add the detergent and swirl it in?
3. What do you think is happening at this step?
4. Does the appearance of the mixture change as you add the meat tenderizer and swirl it in? If so, how?
5. What do you think is happening at this step?
6. Describe the appearance of the mixture just after you added the alcohol.
7. What do you think is happening at this step?
8. What did you see as you twirled the stick at the interface?
9. What do you think is happening at this step?
10. What happens as you slowly pull the stick out of the tube?
11. What does DNA look like?

B.2

Name ______________________________

Date ____________________ Hour ____________

DISCUSSION AND CONCLUSIONS

1. Explain why DNA is so important to study.

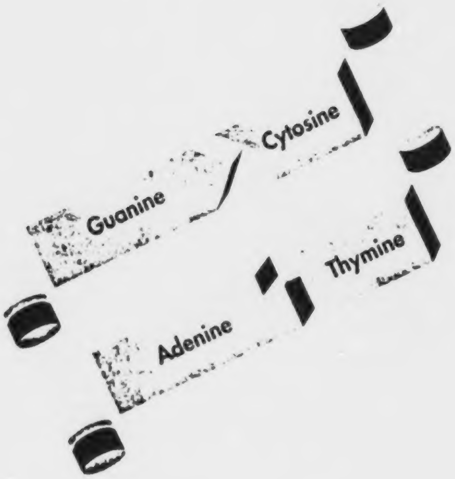
2. Describe in your own words how one isolates DNA from animal tissue.

3. Explain the function of the following reagents: Woolite, Adolph's Meat Tenderizer, and alcohol.

4. What does the DNA look like at the end of the procedure?

CHAPTER 1

DNA: The Hereditary Molecule


Cytosine
Guanine
Thymine
Adenine

SECTION C

What is the Structure of DNA?

C.1

The Puzzle of DNA Structure and Replication

INTRODUCTION

THE FUNCTION OF DNA is based entirely on its structure. A single strand of DNA consists of many individual building blocks called **nucleotides** that are connected, in a long string, by chemical bonds. Each nucleotide contains one of four possible **nitrogenous bases**: either **adenine (A), cytosine (C), guanine (G), or thymine (T)**. In principle, it is possible to construct a DNA strand that contains these four types of nucleotides in any conceivable sequence. In nature, however, DNA exists as a double-stranded molecule in which the two strands that lie side by side interact in a very specific way. The nature of the interactions that occur between these two strands is such that the sequence of nitrogenous bases on one strand always has a predictable relationship to the sequence of nitrogenous bases on its partner strand. This relationship underlies the ability of DNA to serve as the hereditary material and has sometimes been called "the fundamental secret of life."

In this exercise, you will be provided with half of a file folder and a set of puzzle pieces of different shapes and colors that represent the four kinds of nitrogenous bases that are found in all DNA molecules. You will use these pieces to solve a simple puzzle, and from the finished puzzle, you should be able to figure out the nature of the relationship that exists between opposite strands of a double-stranded DNA molecule, and why DNA is so easy to replicate when a cell gets ready to divide.

MATERIALS

For each student or pair of students:
- one half of a file folder
- puzzle pieces

PROCEDURE, PART A

1. Draw a straight line near and parallel to the left hand edge of your piece of file folder.

2. Select ten of your puzzle pieces at random, or in any order that you wish. Place each of these pieces on the paper so that it is letter-side-up and so that its flat end touches the line you have drawn (Fig. 1). This will be the left-hand strand of your DNA puzzle.

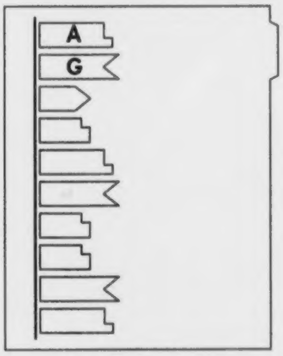


Figure 1

3. Now form the right-hand strand of the DNA by placing the remaining puzzle pieces on the paper so that the non-flat end each of them fits snugly against the non-flat end of one of the pieces in the left-hand strand.

4. Draw a straight line next to the flat ends of the pieces in the right hand strand (Fig. 2).

5. Use your finished puzzle to answer the first four questions on the next page.

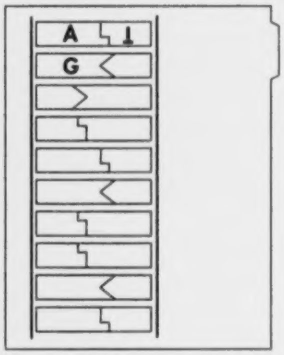


Figure 2

PROCEDURE, PART B

6. Pair up with another person in your class.

7. One person in each pair should put all of his or her puzzle pieces back in the bag (Fig. 3).

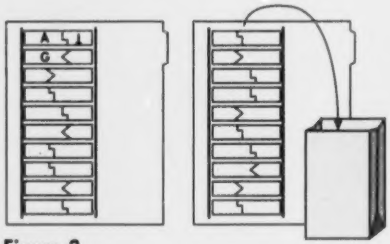


Figure 3

8. Carefully transfer the right-hand strand of the remaining DNA puzzle to the right hand side of the other person's piece of cardboard, leaving the left-hand strand where it is (Fig. 4).

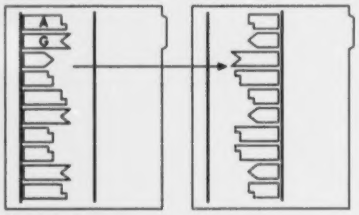


Figure 4

9. Use the puzzle pieces in the bag to build a second DNA strand on each piece of cardboard (Fig. 5).

10. Answer questions 5 and 6 on the next page.

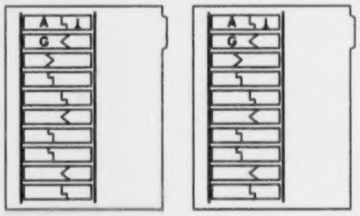


Figure 5

C.1

Name ______________________________

Date ____________________ Hour __________

ANALYSIS AND CONCLUSIONS

1. Do you see any consistent relationship between the DNA bases (puzzle pieces) in one strand of your puzzle and the bases with which they are paired in the other strand? If so, state the nature of the relationship(s) you see.

2. Half of the puzzle pieces that you were given (the A's and G's) were much larger than the other pieces (the C's and T's). Did this size difference cause your DNA model to be significantly wider in some parts than in others? If not, why not?

3. Is there any consistent difference in the way that the pieces in the right-hand strands and the left-hand strands of your model are oriented? If so, what is the difference?

4. How can you account for the fact that no matter which bases were selected for the left-hand strand of a DNA molecule, everyone had just the right pieces left over to assemble a matching right-hand strand?

Do Part B of the puzzle before you answer the next two questions.

5. Are the two DNA puzzles you now have the same or different? How can you account for this?

6. What do you suppose biologists call this process of making two identical double-stranded DNA molecules from one when it occurs in cells?

C.2

The Spiral Staircase

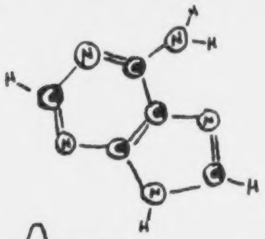
THE SPIRAL STAIRCASE

DNA? IS THAT A GOVERNMENT AGENCY?

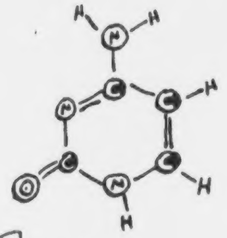
IN 1944, A TEAM OF BIOLOGISTS LED BY OSWALD AVERY MADE A VERY IMPORTANT DISCOVERY: DNA EXTRACTED FROM ONE KIND OF BACTERIUM COULD BE USED TO TRANSFER HERITABLE TRAITS OF THAT BACTERIUM TO A SECOND BACTERIUM! THIS WAS THE FIRST CLEAR INDICATION THAT DNA WAS THE CARRIER OF HEREDITARY INFORMATION. OTHER REPORTS DRAWING THE SAME CONCLUSION SOON FOLLOWED.

BEFORE AVERY, SCIENTISTS HAD PAID LITTLE ATTENTION TO DNA. THEY KNEW IT CONTAINED THE SUGAR DEOXYRIBOSE, PLENTY OF PHOSPHATE AND FOUR BASES.

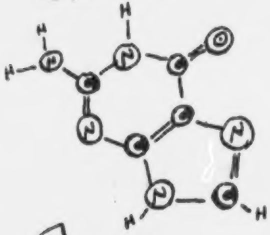
THE FOUR BASES ARE KNOWN AS **A, C, G,** AND **T**, WHICH ARE SHORT FOR:



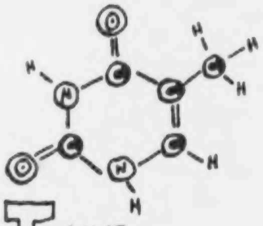
ADENINE



CYTOSINE



GUANINE



THYMINE

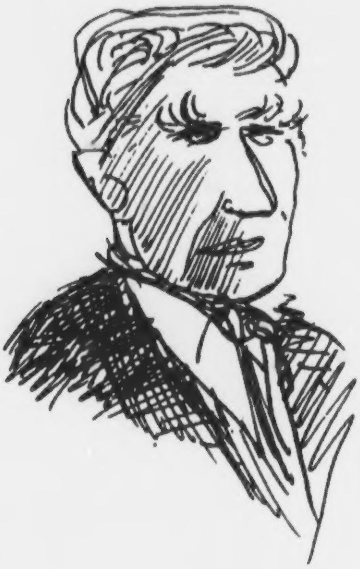
THESE WERE ASSUMED TO BE PRESENT IN EQUAL PROPORTIONS.

Reprinted with permission from Gonick, L. and Whellis, M. (1991).
The Cartoon Guide to Genetics. New York: Harper Collins

C.2

AFTER AVERY, HOWEVER, RESEARCHERS BEGAN TO LOOK MORE CLOSELY...

ERWIN CHARGAFF FOUND.



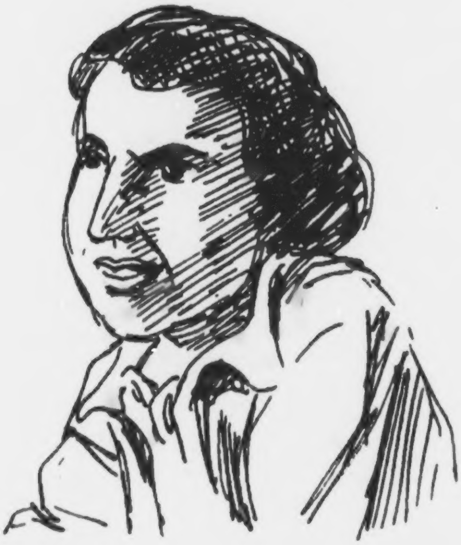
① THE COMPOSITION OF DNA VARIED FROM ONE SPECIES TO ANOTHER, IN PARTICULAR IN THE RELATIVE AMOUNTS OF THE BASES A, C, T, G.

② IN ANY DNA, **THE NUMBER OF A's** WAS **THE SAME AS THE NUMBER OF T's**; SIMILARLY, THE NUMBER OF C's WAS EQUAL TO THE NUMBER OF G's.

WHAT DID THIS MEAN? CHARGAFF COULDN'T SAY...

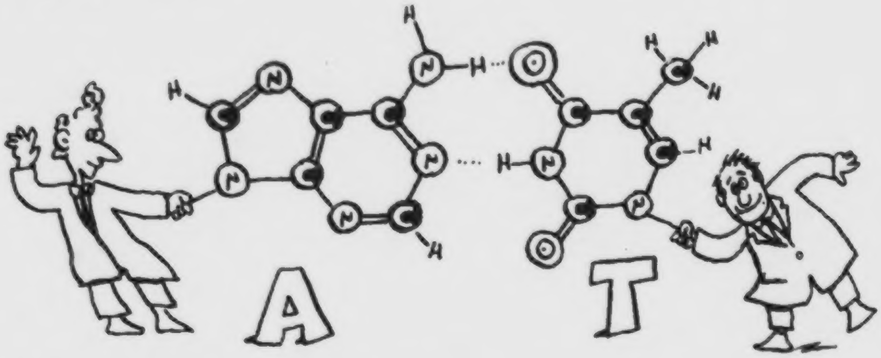
BY STUDYING X-RAY PICTURES OF DNA, **ROSALIND FRANKLIN** WAS ABLE TO SHOW THAT THE DNA MOLECULE PROBABLY HAD THE CORKSCREW SHAPE OF A **HELIX** WITH TWO OR THREE CHAINS...

BUT WAS IT TWO OR THREE...?

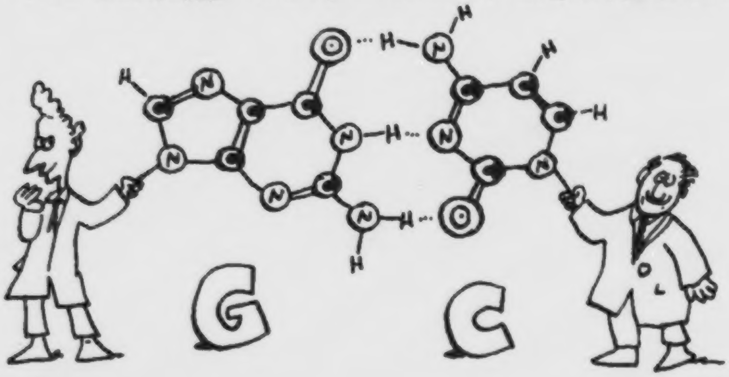


C.2

IN 1952 ***JAMES WATSON*** AND ***FRANCIS CRICK*** CRACKED THE PUZZLE.

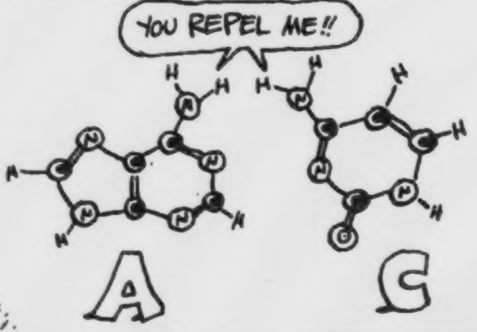


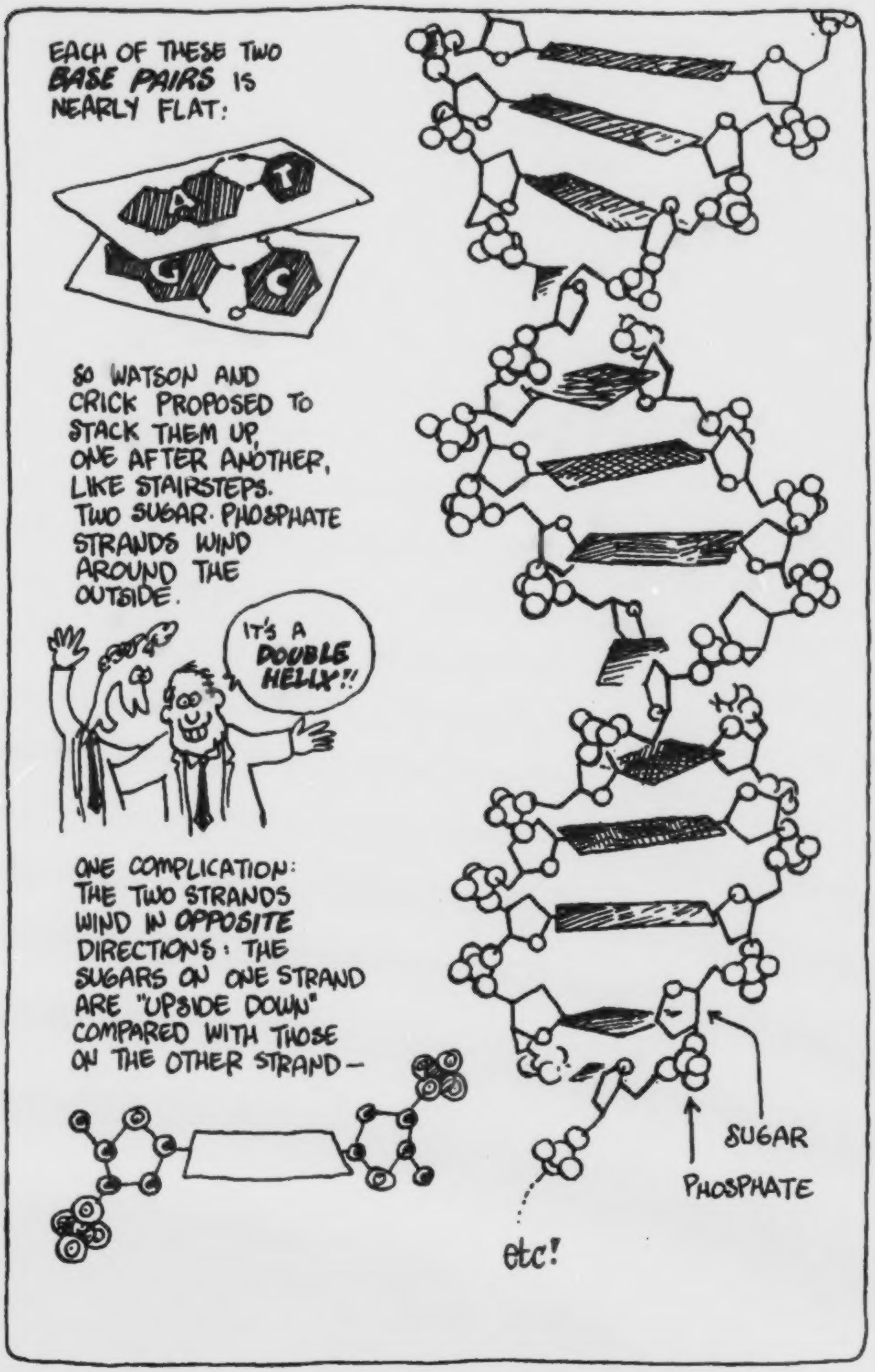
BY PLAYING WITH SCALE-MODEL ATOMS, THEY OBSERVED THAT ***ADENINE*** FITTED TOGETHER WITH ***THYMINE***, WHILE ***GUANINE*** PAIRED NATURALLY WITH ***CYTOSINE***.



EACH BASE PAIR WOULD BE HELD TOGETHER BY ***HYDROGEN BONDING***, A WEAK ATTRACTION THAT MAY OCCUR BETWEEN A HYDROGEN ON ONE MOLECULE AND A NON-HYDROGEN ATOM ON ANOTHER MOLECULE.

IT WAS ALSO CLEAR **A** DID NOT FIT WITH **C**, NOR **G** WITH **T**.





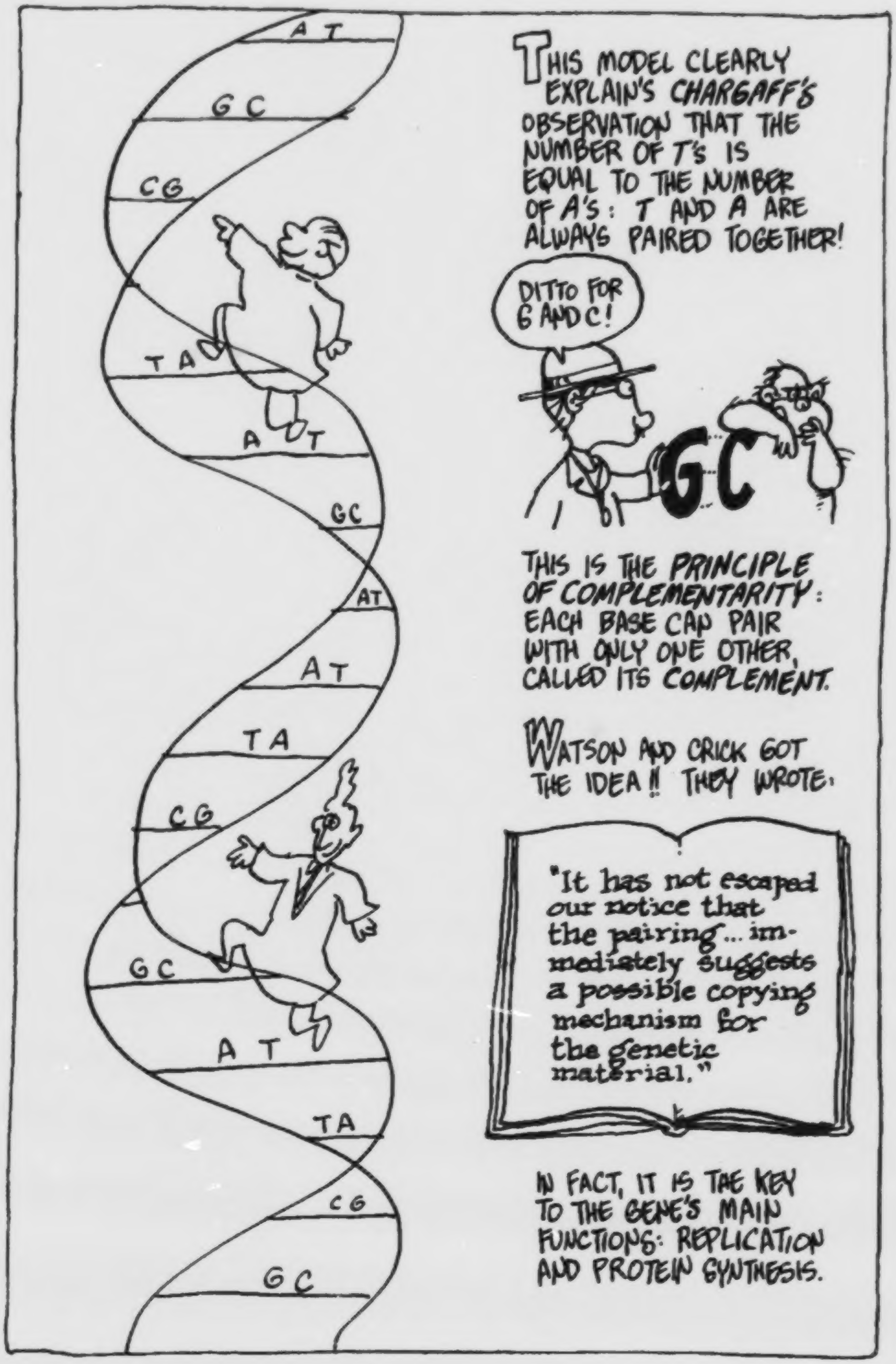
EACH OF THESE TWO ***BASE PAIRS*** IS NEARLY FLAT:

SO WATSON AND CRICK PROPOSED TO STACK THEM UP, ONE AFTER ANOTHER, LIKE STAIRSTEPS. TWO SUGAR-PHOSPHATE STRANDS WIND AROUND THE OUTSIDE.

ONE COMPLICATION: THE TWO STRANDS WIND IN ***OPPOSITE*** DIRECTIONS: THE SUGARS ON ONE STRAND ARE "UPSIDE DOWN" COMPARED WITH THOSE ON THE OTHER STRAND—

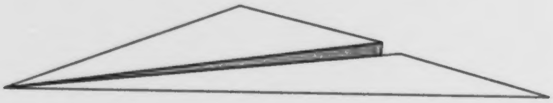
A
T
G
C
IT'S A **DOUBLE HELIX**!!
SUGAR
PHOSPHATE
etc!

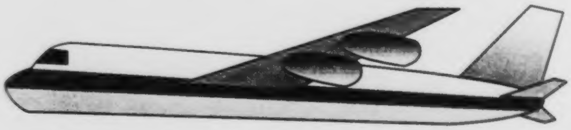
C.2


AT
GC
CG
TA
AT
GC
AT
AT
TA
CG
GC
AT
TA
CG
GC
THIS MODEL CLEARLY EXPLAIN'S CHARGAFF'S OBSERVATION THAT THE NUMBER OF T'S IS EQUAL TO THE NUMBER OF A'S: T AND A ARE ALWAYS PAIRED TOGETHER!
DITTO FOR G AND C!
GC
THIS IS THE PRINCIPLE OF COMPLEMENTARITY: EACH BASE CAN PAIR WITH ONLY ONE OTHER, CALLED ITS COMPLEMENT.
WATSON AND CRICK GOT THE IDEA!! THEY WROTE:
"It has not escaped our notice that the pairing... immediately suggests a possible copying mechanism for the genetic material."
IN FACT, IT IS THE KEY TO THE GENE'S MAIN FUNCTIONS: REPLICATION AND PROTEIN SYNTHESIS.

C.3

What is a Model? And What is it Good For?





AS WE GREW UP, most of us played with various models: model cars, model planes, model houses (doll houses), model people (dolls), etc. Not all models are designed for childhood play, however. Models serve important functions in many areas of adult life. Engineers, for example, evaluate various possible airplane designs by building scale models and examining how they behave in a wind tunnel (which is itself a model of the atmosphere).

Models are usually highly simplified relative to the objects that they portray, and often several different models, simplified in different ways, are required to study different aspects of the same object. For example, the engineer who is designing the seats for a commercial airliner requires a very different kind of model than does the engineer who is designing the wings. Neither of these models would necessarily be more correct than the other; they would merely be simplified in different ways in order to serve different purposes.

DNA MODELS

Scientists find models of various kinds of molecules very useful for visualizing how their parts fit together and for predicting what their properties should be. Indeed, it was only when two biologists by the names of Watson and Crick built the first fairly accurate scale model of DNA that it became clear what the structure of real DNA molecules must be.

In the next exercise, you will build a much simpler DNA model than Watson and Crick did in order to visualize certain very simple aspects of its structure. But as you progress in the study of biology, you will probably encounter several other kinds of DNA models that are constructed so as to reveal other, more detailed aspects of DNA structure and function.

C.4

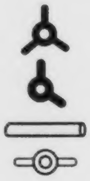
Building a Three-Dimensional DNA Model

MATERIALS

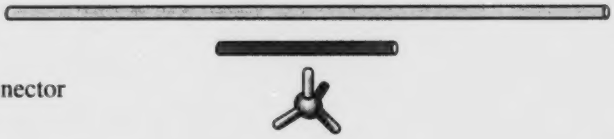
For each student or pair of students: Use the following list to make sure that your model-building kit contains the correct number of pieces of each type. Then use it to determine what each of these pieces represents.

Short straws representing the nitrogenous bases:
- 3 blue straws = A (adenine)
- 3 red straws = T (thymine)
- 3 green straws = G (guanine)
- 3 gray straws = C (cytosine)

Pieces used to build the sugar-phosphate ladders:
- 12 black connectors = sugars (deoxyribose)
- 12 red connectors = phosphate groups
- 24 yellow straws = sugar-to-phosphate bonds
- 6 white connectors = hydrogen bonds



Pieces used to build the stand for the model:
- 1 long gray straw
- 3 medium-length green straws
- 1 four-prong black or silver connector



PROCEDURE

1. Make 12 nucleotides as shown in figure 1. Each nucleotide will require one deoxyribose sugar, two yellow sugar-to-phosphate bonds, one red phosphate group, and one nitrogenous base (either red, green, blue, or gray).

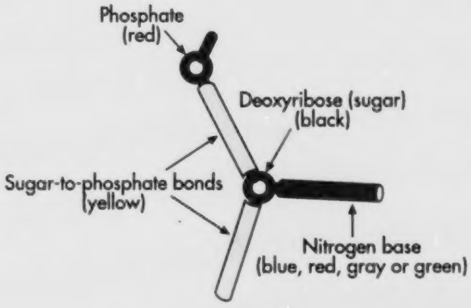

Phosphate (red)
Deoxyribose (sugar) (black)
Sugar-to-phosphate bonds (yellow)
Nitrogen base (blue, red, gray or green)

Figure 1: Making a nucleotide

2. After you have made all the nucleotides you can, use the white connectors (hydrogen bonds) to join them in pairs according to the base-pairing rules (A to T and G to C), as in figure 2.

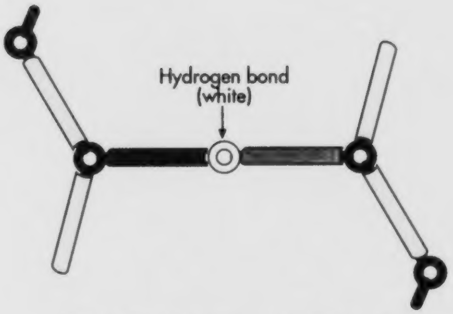

Hydrogen bond
(white)

Figure 2: Making a nucleotide pair

3. After you have finished forming the nucleotide pairs, use the sugar-phosphate bonds to join them (fig. 3), in any order you want.

4. Note that the sugar-phosphate chain on the left side has a red phosphate group at the bottom (its 5′ end) but not at the top (its 3′ end) and that the sugar-phosphate chain on the right side is just the opposite. This is one of the ways that enzymes that interact with DNA molecules can tell the two strands apart.

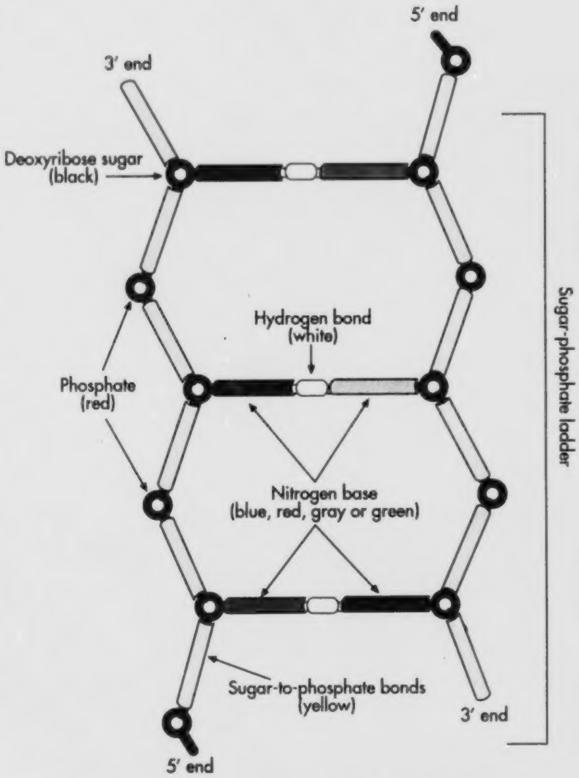

5′ end
3′ end
Deoxyribose sugar
(black)
Hydrogen bond
(white)
Phosphate
(red)
Nitrogen base
(blue, red, gray or green)
Sugar-to-phosphate bonds
(yellow)
3′ end
5′ end
Sugar-phosphate ladder

Figure 3: Joining the nucleotide pairs

5. Build the stand for your model using the one long gray straw, the three medium-length green straws and the four-pronged black connector.

6. Slide the white hydrogen bonds that run down the middle of your model onto the stand.

7. To get all of your model to fit on the stand, you will need to twist it counterclockwise at the top (fig. 4). Now you have a representation of the famous double-helix of DNA.

8. Answer the questions on the next page.

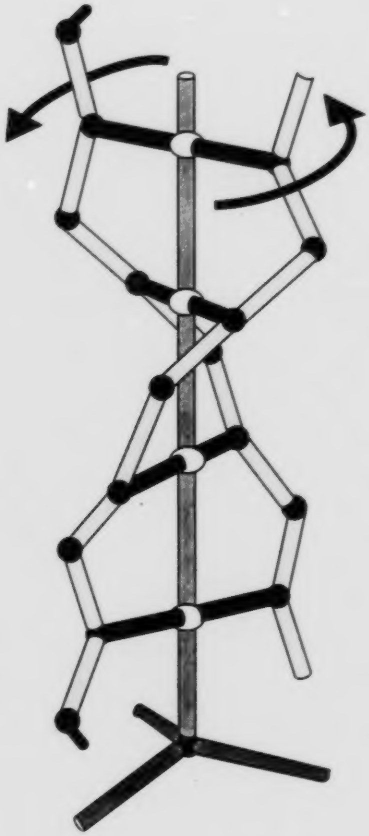


Figure 4: The completed model on a stand

C.4

Name ______________________________

Date ________________ Hour __________

ANALYSIS AND CONCLUSIONS

YOU HAVE NOW built two models of double-stranded DNA: the flat one in the puzzle at the beginning of this exercise that you used to deduce the base-pairing rules and how DNA is replicated (which we will call "model A"), and the one you have just built with straws and connectors (which we will call "model B"). As mentioned earlier, often two models of the same thing will be simplified in different ways to emphasize different features of the object they are representing.

1. What feature or features of a double-stranded DNA molecule are represented better in model A than in model B?

   ____

   ____

   ____

2. What feature or features of a double-stranded DNA molecule are represented better in model B than in model A?

   ____

   ____

   ____

3. What feature or features of a double-stranded DNA molecule that you read about in the excerpt from the Cartoon Guide to Genetics are not well represented in either model A or model B?

   ____

   ____

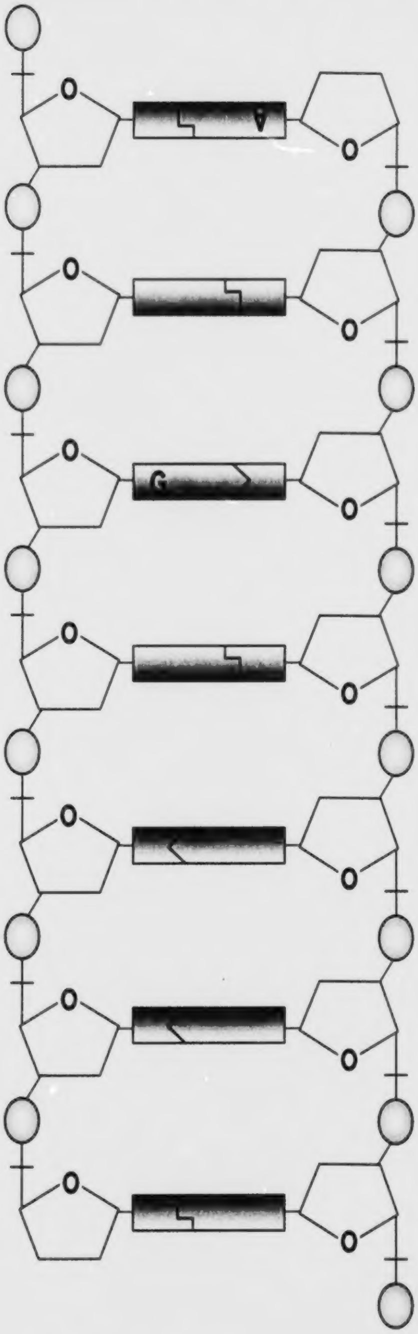
   ____

C.5

Name ______________________________

Date ________________ Hour __________

DNA Model Questions



THE DIAGRAM ON THE left represents an untwisted, double-stranded DNA molecule.

1. Label each sugar group on the diagram with a letter S.
2. Label each phosphate group with a letter P.
3. One adenine (A) and one guanine (G) have already been labeled. Label the rest of the nitrogenous bases.
4. Circle one nucleotide. What three things go together to make a nucleotide?

   ____

   ____

   ____

5. The sides of the DNA ladder are made up of alternating ________________ and ________________ groups.
6. The rungs of the DNA ladder are made up of ________________
7. A is always paired with ____________
8. G is always paired with ____________
9. Paired bases are held together by weak bonds called ____________ bonds.
10. When the DNA ladder twists the way it normally does, the shape of the molecule is called a ________________

C.6

Name ____________________

Date ______________ Hour ________

DNA Word Search

FIND AND CIRCLE the words that go with each of the clues given below. Then write the answers on the lines next to the clues.

I	J	Y	R	L	W	X	B	V	X	M	I	U	R	W	Y	T	Q	W	D
U	S	P	G	P	Y	J	W	L	S	V	Q	E	H	B	K	D	N	I	I
B	E	L	D	K	E	Z	P	H	O	S	P	H	A	T	E	L	C	T	U
N	S	N	P	E	E	D	Y	W	B	L	T	T	Q	O	U	A	S	H	K
B	A	E	I	N	Y	E	O	X	I	F	C	H	X	N	C	Q	Y	J	R
E	B	D	A	S	Z	K	M	C	R	R	Q	L	S	I	D	D	G	A	T
O	S	D	B	R	O	H	A	K	C	P	R	M	E	P	R	N	G	D	I
C	U	A	W	M	O	T	C	J	H	I	X	L	G	O	L	U	L	E	S
Z	O	L	W	N	I	V	Y	N	B	N	C	N	G	V	S	B	F	N	L
H	N	D	V	O	O	L	D	C	U	U	K	E	N	E	W	I	S	I	Q
T	E	E	N	R	X	O	S	C	N	Y	N	U	N	I	S	N	N	N	L
Q	G	T	A	E	B	E	L	O	F	B	Q	I	V	E	B	O	Q	E	I
T	O	S	D	O	G	E	B	J	O	E	N	I	M	Y	H	T	B	N	X
X	R	I	S	A	O	I	W	N	P	A	L	B	S	P	D	Q	Q	N	R
C	T	W	B	T	R	S	D	G	U	A	H	B	J	Z	Z	Y	F	O	P
V	I	T	I	Y	S	S	T	G	M	T	S	R	A	U	H	S	X	A	V
E	N	D	X	B	A	S	E	P	A	I	R	X	Y	Z	O	C	Y	N	Y
D	E	O	Y	E	Y	O	W	X	I	L	E	H	E	L	B	U	O	D	Q
I	E	J	S	E	A	I	E	Q	V	W	Q	X	M	L	C	B	X	P	V
D	A	X	F	M	N	H	G	K	N	X	I	I	A	F	R	R	G	M	Y

1. The nitrogenous base A ____________
2. The nitrogenous base C ____________
3. The nitrogenous base G ____________
4. The nitrogenous base T ____________
5. The genetic material inside all cells __________ (abbreviation)
6. The full name for DNA ____________ ______________________________
7 The scientific name for the shape of the DNA molecule ______________ ______________________________ (two words)
8. The arrangement of two bases in the DNA molecule forms a ____________ ____________________ (two words)
9. The name of the bonds that hold the two strands of DNA together (between the bases) ____________ (two words)
10. Pairs of these molecules form the steps or rungs in the DNA molecule ______ ______________________________ (two words)
11. This subunit of DNA has three parts: a phosphate, a sugar and a nitrogenous base ________________________
12. The long backbones of the DNA molecule are made of alternating sugar and ________________________ bonds.
13. This process occurs when DNA makes a copy of itself __________________

CHAPTER 1

DNA: The Hereditary Molecule

SECTION D

What Does DNA Do?

D.1

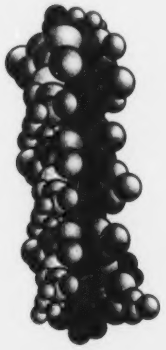
DNA Codes For Proteins

PROTEINS DO THE nitty-gritty jobs of every living cell. **Proteins** are the molecules that give structure and shape to living cells and that carry out all of the chemical reactions necessary for life. The importance of DNA is that it contains the information that is used to make all of the proteins on which life depends.

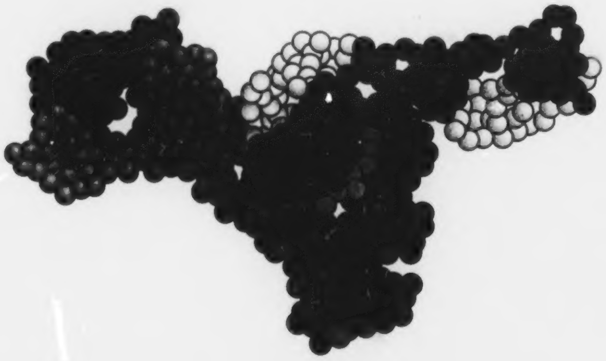
The proteins whose structures are specified by our DNA do more than carry out all of the reactions necessary to keep our cells alive; they also digest our food for us, influence how we will respond to infections, and determine what color our eyes, hair and skin will be.

Proteins are made of long strings of individual building blocks known as **amino acids.** Amino acids come in 20 different kinds that are all slightly different from one another chemically. It is not important for us to understand the details of the chemical differences that distinguish these 20 kinds of amino acids. But it is important to realize that because of these differences, the structure and function of every protein depends on the sequence in which the various kinds of amino acids are strung together. A protein may contain many hundred amino acids. But if just one of these amino acid is changed, the function of the protein might change drastically.

A gene, which is a functional unit of DNA, carries coded information indicating the precise sequence in which amino acids should be strung together to make one particular kind of protein that will play one particular role in the life of the cell.



The elongated shape of collagen protein, shown in this molecular model, allows collagen to provide structural support to cells and organs.



Antibodies, such as this human immunoglobulin, recognize and attack viruses, bacteria, and other foreign substances.

D.1

There are several different categories of proteins. Most proteins function as **enzymes** to regulate the speed of particular chemical reactions. Every cell contains hundreds of different enzymes that work together to release energy from food molecules and to use energy to build new cellular materials. All enzymes are proteins. But not all proteins are enzymes. **Structural proteins,** as the name implies, provide structural support inside and outside cells and for the body as a whole. **Defense proteins** are an assortment of proteins in the blood that recognize and fight off foreign invaders, such as bacteria and viruses. **Transport proteins** are used to carry various molecules into and out of cells as well as through the body. The table on the next page gives a few examples of these and other kinds of proteins.

Every aspect of life depends on proteins. In the next set of activities, you will learn about the processes a cell uses to convert the coded information in a bit of DNA (a gene) into a particular kind of protein.

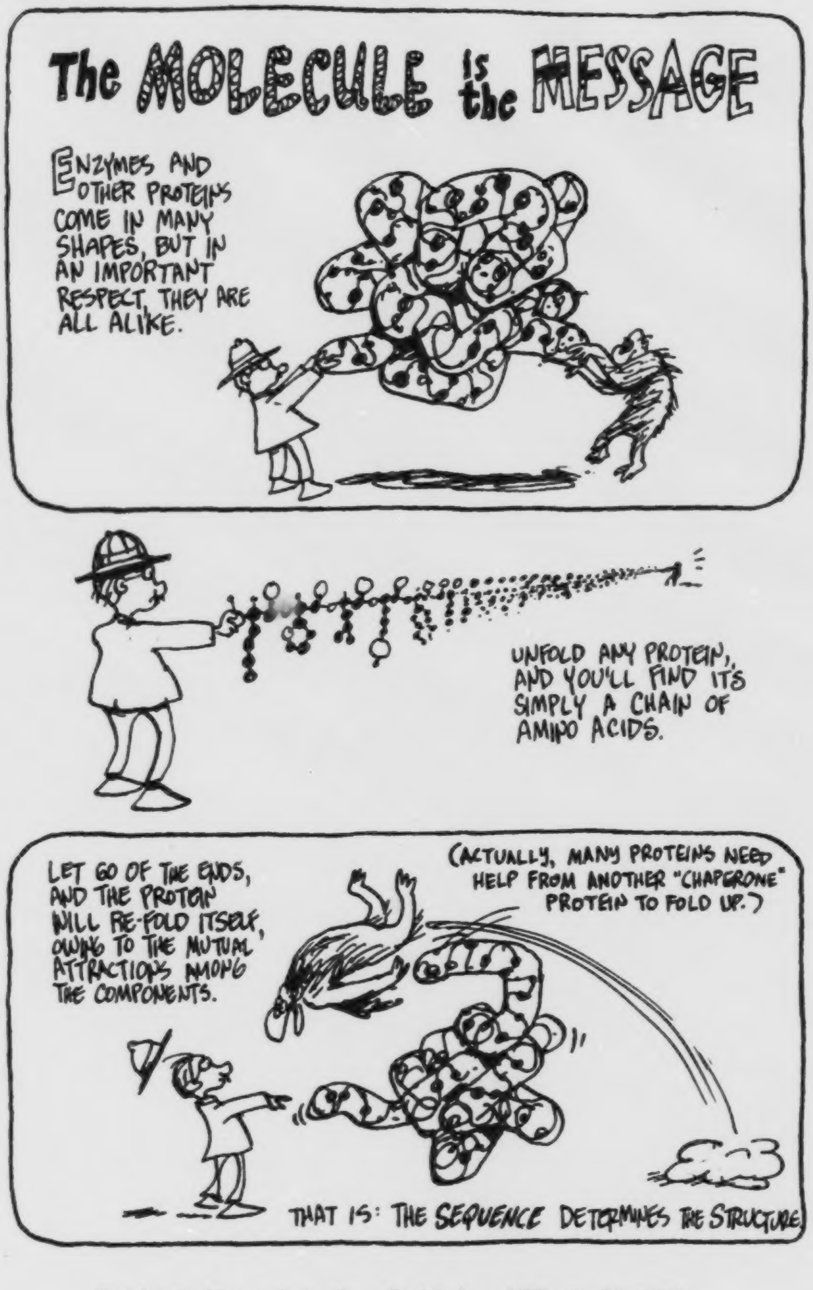
D.1

A FEW HUMAN PROTEINS AND THEIR FUNCTIONS

Protein	Type	What it does
hemoglobin	a red, iron-containing, transport protein	• makes your blood red • picks up oxygen from the air in your lungs and releases it to cells elsewhere in your body • if you don't make enough hemoglobin, or you make a type that doesn't carry oxygen well, you will be anemic
insulin	a hormone	• tells your cells when to remove sugar from your blood after a meal • if you don't make enough insulin, or if your cells do not respond to it well, the concentration of sugar in your blood will get too high, and you will have diabetes
antibody	a defense protein	• fights infections from bacteria and viruses • vaccination triggers production of antibodies that will fight a particular kind of infection • if you couldn't make any antibodies, you would have been dead long ago!
lactase	an enzyme in your digestive tract	• digests lactose (milk sugar) in milk and dairy products into simpler sugars • if you don't make enough lactase, you get diarrhea when you drink milk or eat ice cream or other diary products • but you can buy lactase pills to swallow before eating dairy products
collagen	a structural protein	• provides the tough structural framework of your skin, bones, tendons, and cartilage
keratin	a structural protein	• provides the resistant outer layer of your skin and the tough structural material of your hair and nails
myosin	a structural protein that is also an enzyme	• uses energy derived from food — and works together with actin — to cause muscles to contract • you can increase the myosin content of your muscles with exercise

D.2

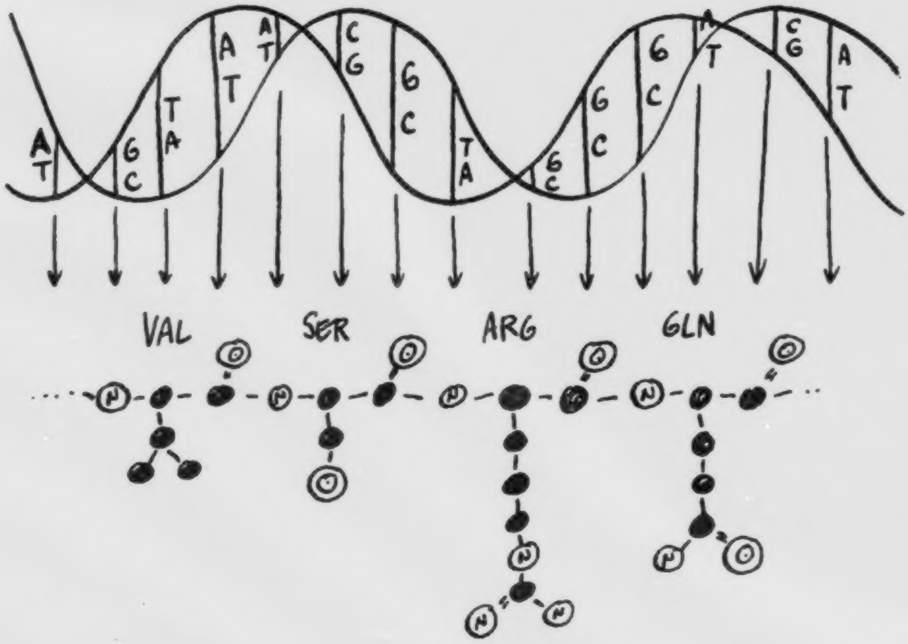
How DNA Codes For Proteins


The MOLECULE is the MESSAGE
ENZYMES AND OTHER PROTEINS COME IN MANY SHAPES, BUT IN AN IMPORTANT RESPECT, THEY ARE ALL ALIKE.
UNFOLD ANY PROTEIN, AND YOU'LL FIND IT'S SIMPLY A CHAIN OF AMINO ACIDS.
LET GO OF THE ENDS, AND THE PROTEIN WILL RE-FOLD ITSELF, OWING TO THE MUTUAL ATTRACTIONS AMONG THE COMPONENTS.
(ACTUALLY, MANY PROTEINS NEED HELP FROM ANOTHER "CHAPERONE" PROTEIN TO FOLD UP.)
THAT IS: THE SEQUENCE DETERMINES THE STRUCTURE

Reprinted with permission from Gonick, L. and Wheelis, M. (1991).
The Cartoon Guide to Genetics. New York: Harper Collins Publishers

D.2

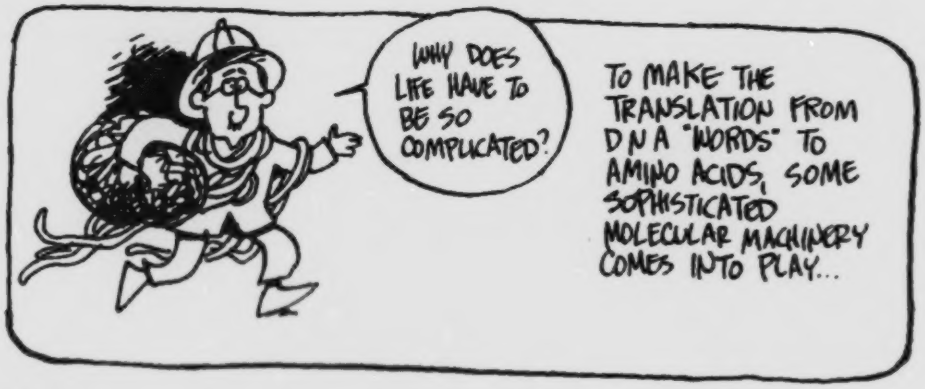
IN VIEW OF THE RELATIONSHIP BETWEEN GENES AND PROTEINS, THIS SUGGESTS THAT THE *SEQUENCE* OF DNA MUST SOMEHOW PARALLEL OR REFLECT THE *SEQUENCE* OF THE PROTEIN.

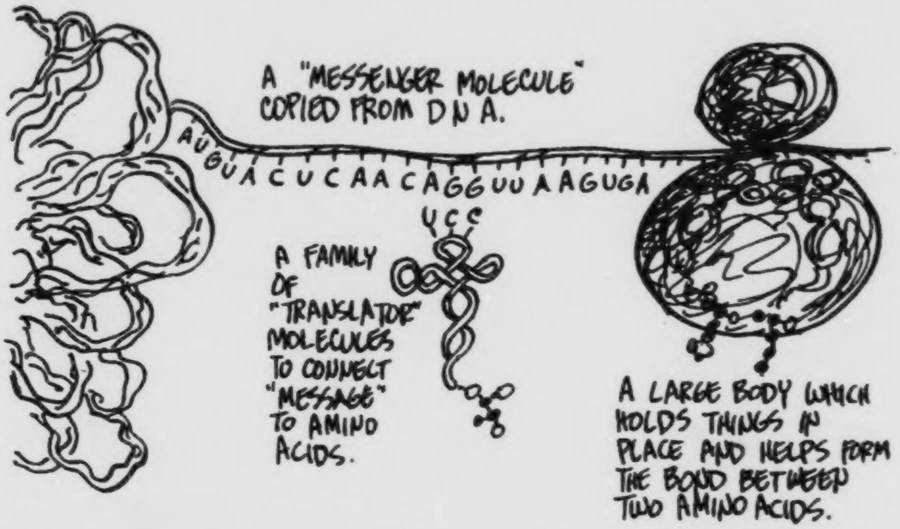

A T
G C
T A
A T
A T
C G
G C
T A
G C
G C
G C
A T
C G
A T
VAL
SER
ARG
GLN

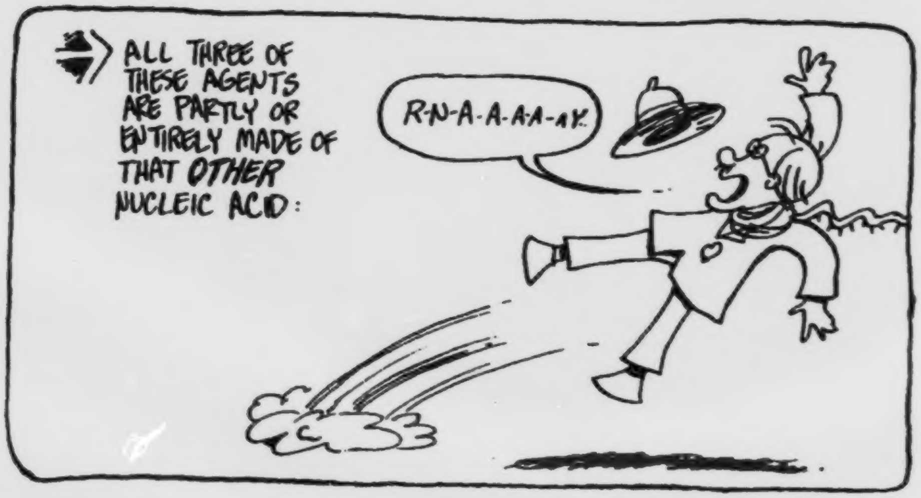
THE MAIN IDEA:

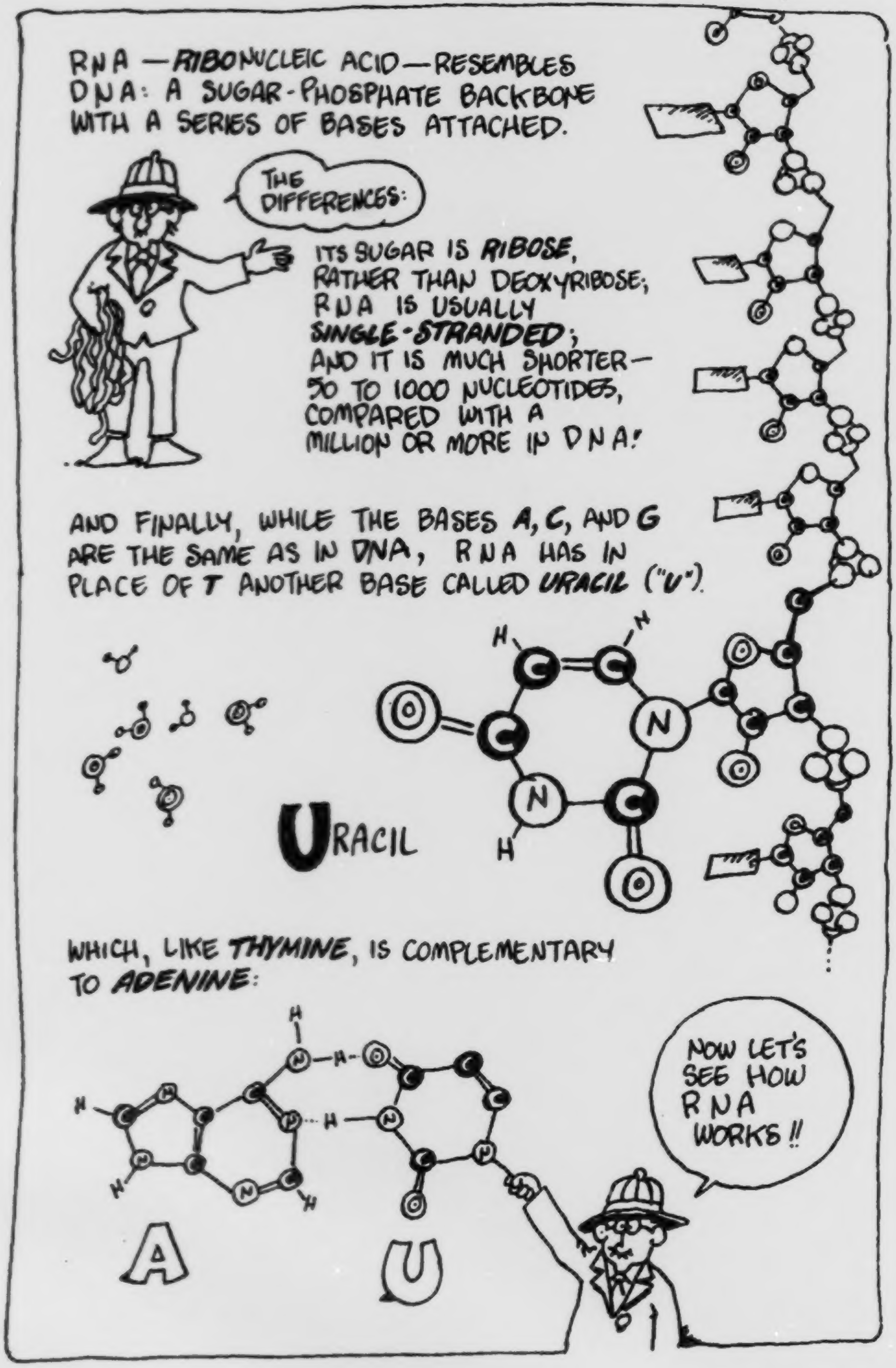
The sequence of base pairs may be thought of as a series of "words" specifying the order of amino acids in each protein.

D.2


WHY DOES LIFE HAVE TO BE SO COMPLICATED?
TO MAKE THE TRANSLATION FROM DNA "WORDS" TO AMINO ACIDS, SOME SOPHISTICATED MOLECULAR MACHINERY COMES INTO PLAY...

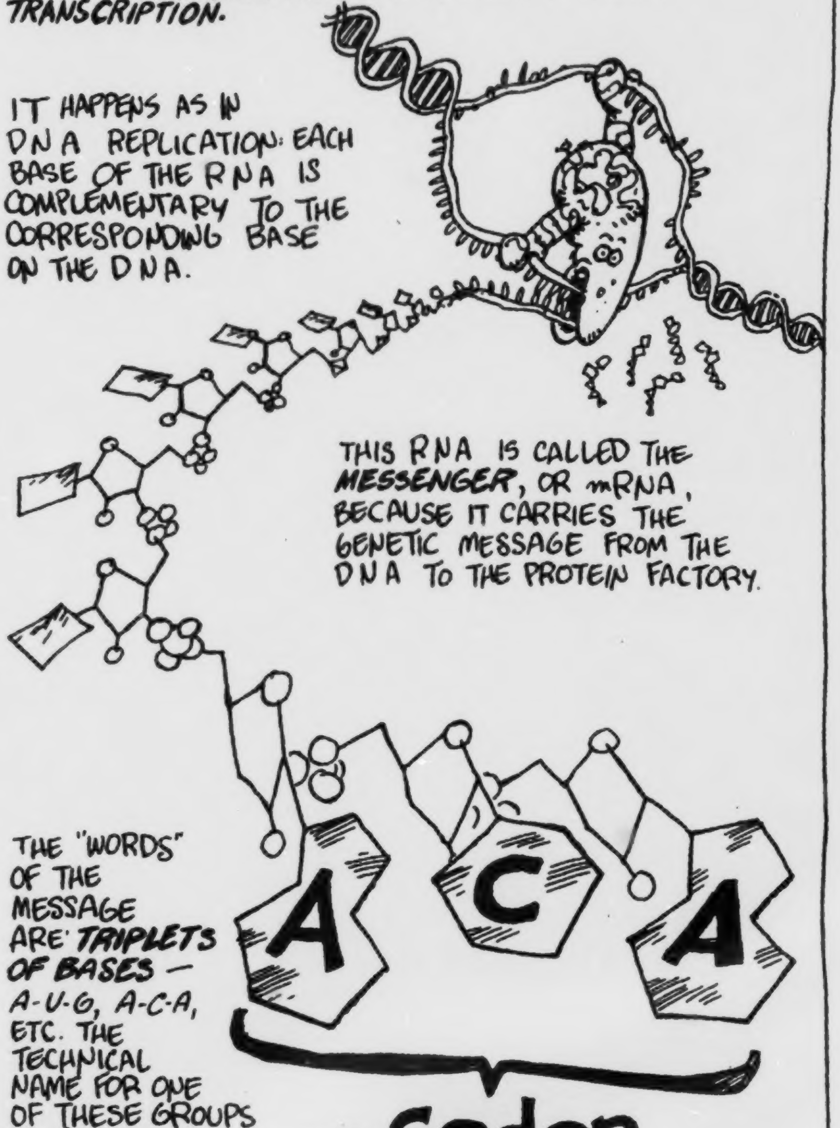

A "MESSENGER MOLECULE" COPIED FROM DNA.
AUGUACUCAACAGGUUAAGUGA
UCC
A FAMILY OF "TRANSLATOR" MOLECULES TO CONNECT "MESSAGE" TO AMINO ACIDS.
A LARGE BODY WHICH HOLDS THINGS IN PLACE AND HELPS FORM THE BOND BETWEEN TWO AMINO ACIDS.


ALL THREE OF THESE AGENTS ARE PARTLY OR ENTIRELY MADE OF THAT *OTHER* NUCLEIC ACID:
R-N-A-A-A-A-AY.


RNA —RIBONUCLEIC ACID—RESEMBLES DNA: A SUGAR-PHOSPHATE BACKBONE WITH A SERIES OF BASES ATTACHED.
THE DIFFERENCES:
ITS SUGAR IS RIBOSE, RATHER THAN DEOXYRIBOSE; RNA IS USUALLY SINGLE-STRANDED; AND IT IS MUCH SHORTER—50 TO 1000 NUCLEOTIDES, COMPARED WITH A MILLION OR MORE IN DNA!
AND FINALLY, WHILE THE BASES A, C, AND G ARE THE SAME AS IN DNA, RNA HAS IN PLACE OF T ANOTHER BASE CALLED URACIL ("U").
URACIL
WHICH, LIKE THYMINE, IS COMPLEMENTARY TO ADENINE:
A
U
NOW LET'S SEE HOW RNA WORKS!!

PROTEIN SYNTHESIS BEGINS WHEN A REGION OF DNA IS TEASED APART AND A MOLECULE OF RNA IS BUILT ALONG ONE STRAND BY AN ENZYME CALLED ***RNA POLYMERASE.*** THIS PROCESS IS CALLED ***TRANSCRIPTION.***

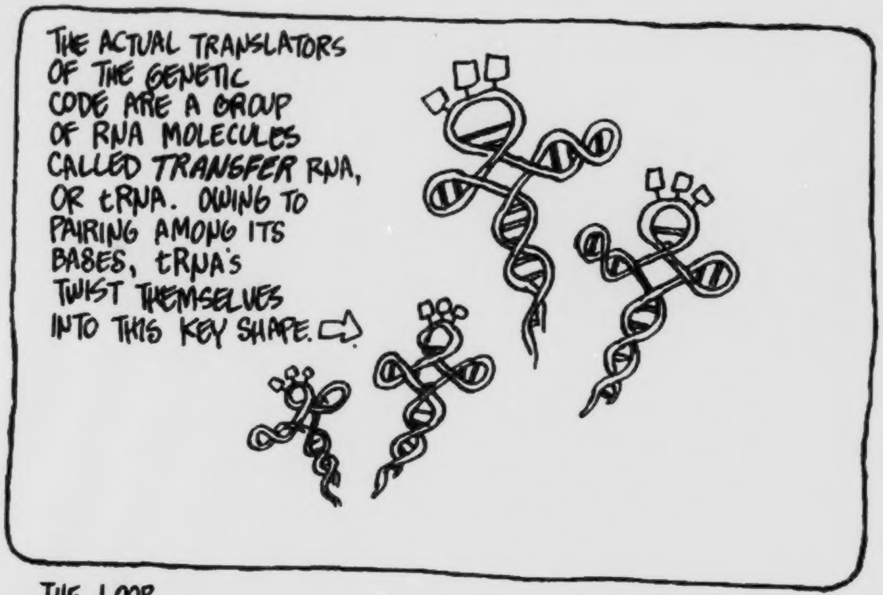
IT HAPPENS AS IN DNA REPLICATION: EACH BASE OF THE RNA IS COMPLEMENTARY TO THE CORRESPONDING BASE ON THE DNA.



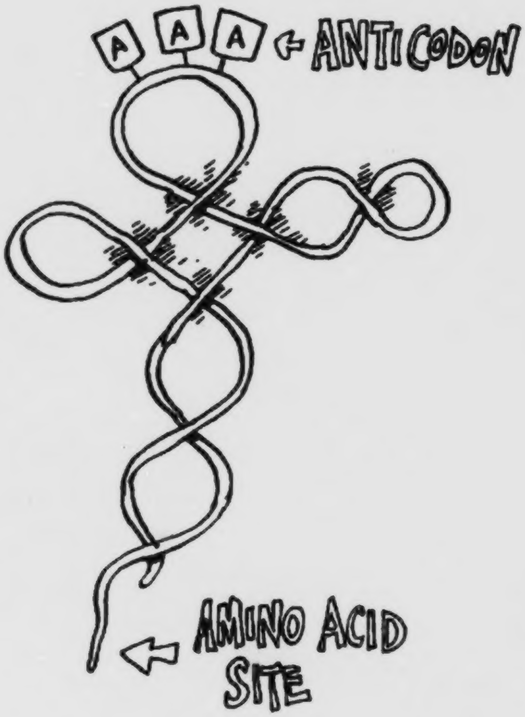
THIS RNA IS CALLED THE ***MESSENGER***, OR mRNA, BECAUSE IT CARRIES THE GENETIC MESSAGE FROM THE DNA TO THE PROTEIN FACTORY.

THE "WORDS" OF THE MESSAGE ARE ***TRIPLETS OF BASES*** — *A-U-G, A-C-A,* ETC. THE TECHNICAL NAME FOR ONE OF THESE GROUPS IS A → **Codon**

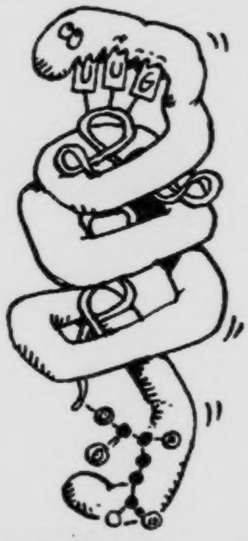
D.2


THE ACTUAL TRANSLATORS OF THE GENETIC CODE ARE A GROUP OF RNA MOLECULES CALLED *TRANSFER* RNA, OR tRNA. OWING TO PAIRING AMONG ITS BASES, tRNA'S TWIST THEMSELVES INTO THIS KEY SHAPE. ⇨

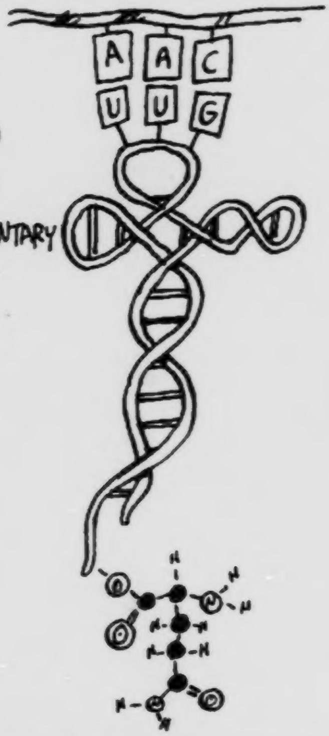
THE LOOP END OF tRNA HAS THREE UNPAIRED BASES. THIS "*ANTICODON*" MAY BIND WITH THE COMPLEMENTARY CODON OF mRNA. AT THE "TAIL" END OF tRNA IS A SITE FOR ATTACHING A SINGLE AMINO ACID.

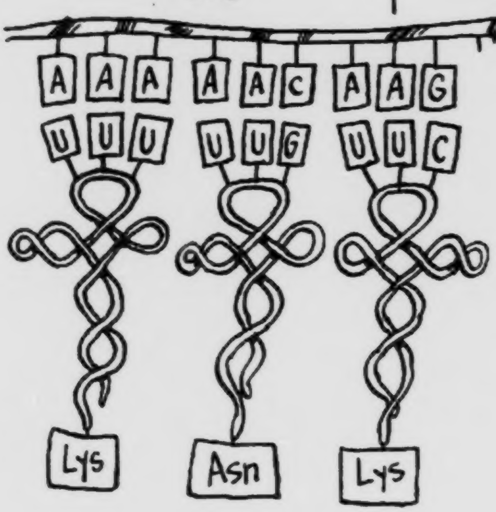

A
A
A
ANTICODON
AMINO ACID SITE

FOR EACH ANTICODON, THERE IS AN ENZYME WHICH RECOGNIZES IT AND ATTACHES THE APPROPRIATE AMINO ACID TO ITS *t*RNA.



ONCE THEY ARE LINKED, THE ANTICODON BINDS TO THE COMPLEMENTARY CODON OF MESSAGE.





SCHEMATICALLY, THIS IS THE WAY A STRING OF BASES IS TRANSLATED INTO A SEQUENCE OF AMINO ACIDS.

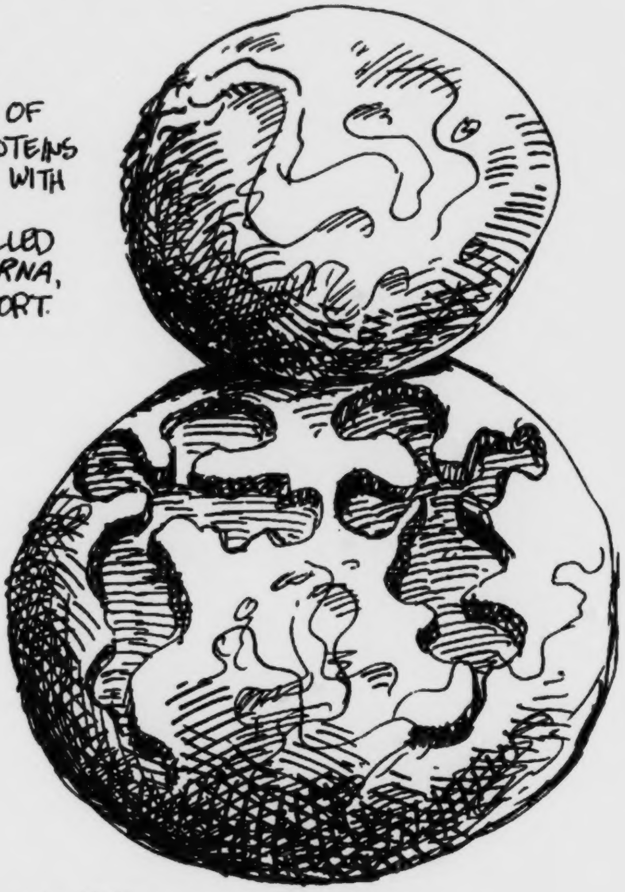
HOWEVER,

THE CELL NEEDS ONE MORE PIECE OF EQUIPMENT TO MAKE IT WORK: THE *RIBOSOME*.

HOW PROTEINS ARE MADE

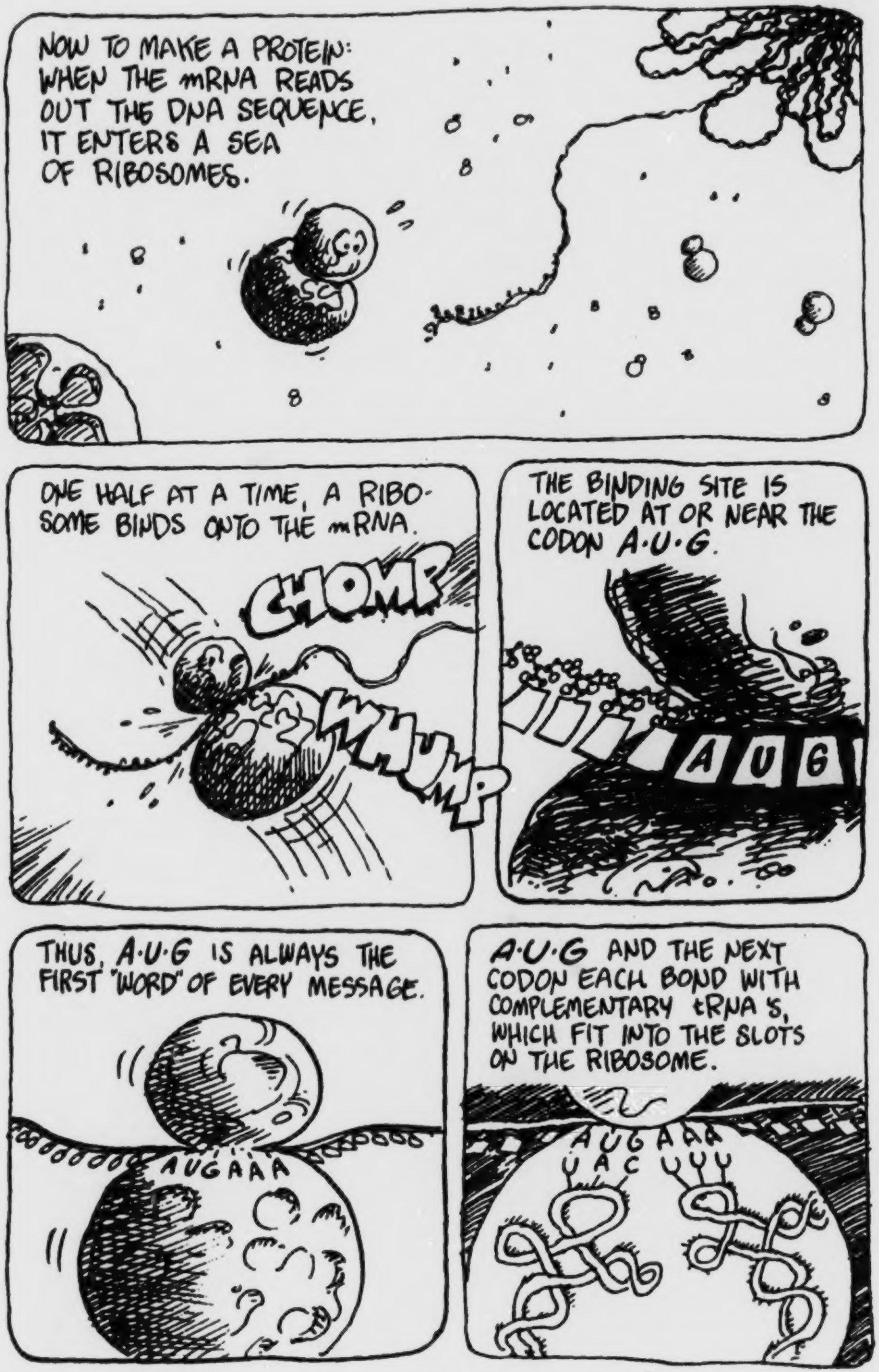
THE FINAL INGREDIENT IN THE PROTEIN-MAKING APPARATUS IS AN OBJECT THAT HOLDS EVERYTHING IN PLACE.

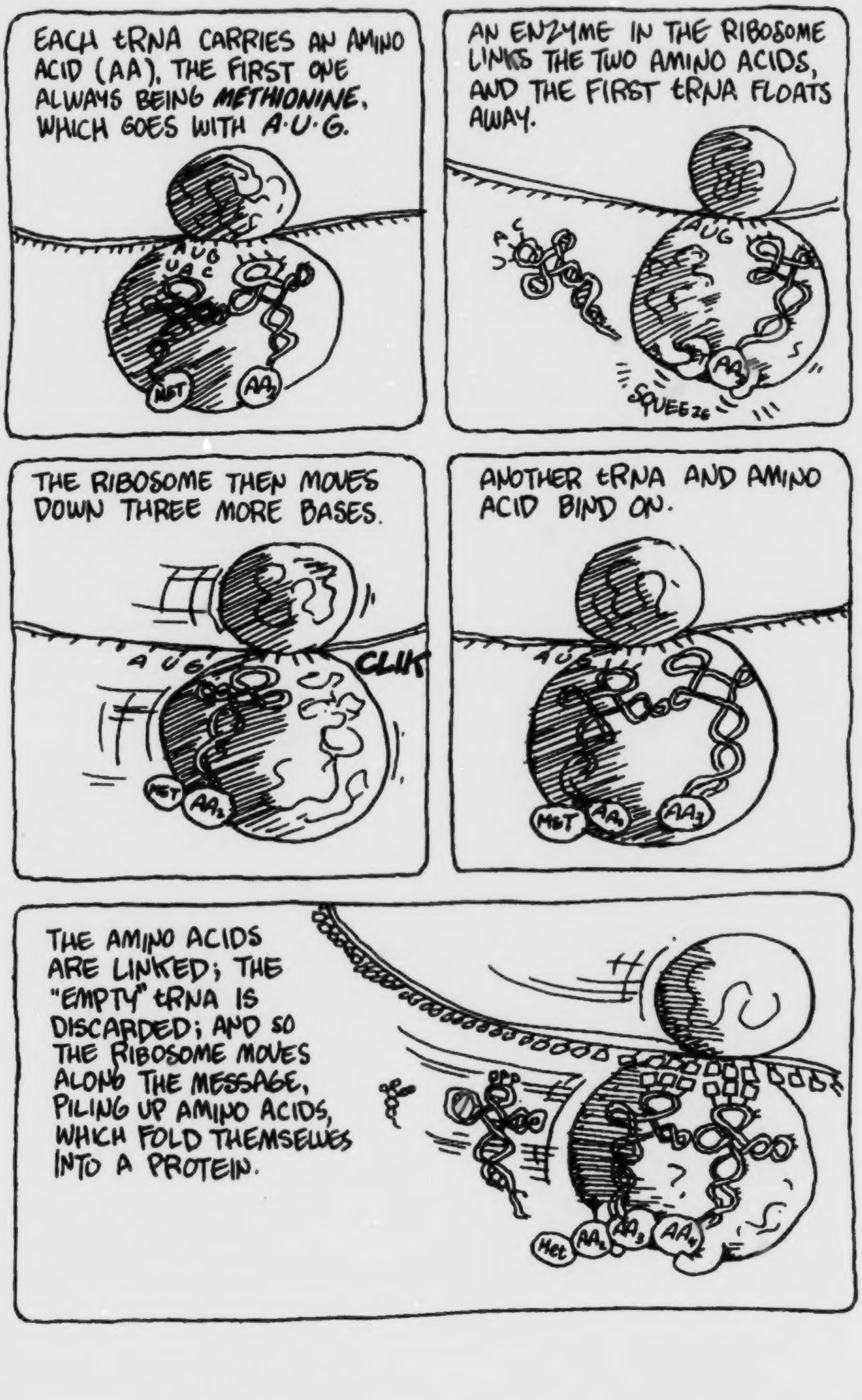
THIS IS THE ***RIBOSOME***, A DOUBLE BALL OF ABOUT 50 PROTEINS WRAPPED UP WITH RNA. THIS RNA IS CALLED ***RIBOSOMAL RNA***, rRNA FOR SHORT.



THE RIBOSOME HAS TWO SLOTS IN WHICH MOLECULES OF tRNA CAN FIT SNUGLY.

D.2


NOW TO MAKE A PROTEIN: WHEN THE mRNA READS OUT THE DNA SEQUENCE, IT ENTERS A SEA OF RIBOSOMES.
ONE HALF AT A TIME, A RIBOSOME BINDS ONTO THE mRNA.
CHOMP
WHUMP
THE BINDING SITE IS LOCATED AT OR NEAR THE CODON A·U·G.
A U G
THUS, A·U·G IS ALWAYS THE FIRST "WORD" OF EVERY MESSAGE.
AUGAAA
A·U·G AND THE NEXT CODON EACH BOND WITH COMPLEMENTARY tRNA'S, WHICH FIT INTO THE SLOTS ON THE RIBOSOME.
AUGAAA
UAC UUU


EACH tRNA CARRIES AN AMINO ACID (AA), THE FIRST ONE ALWAYS BEING METHIONINE, WHICH GOES WITH A·U·G.
AUG
UAC
MET
AA2
AN ENZYME IN THE RIBOSOME LINKS THE TWO AMINO ACIDS, AND THE FIRST tRNA FLOATS AWAY.
AUG
UAC
AA2
SQUEEZ
THE RIBOSOME THEN MOVES DOWN THREE MORE BASES.
AUG
CLIK
MET
AA2
ANOTHER tRNA AND AMINO ACID BIND ON.
AUG
MET
AA2
AA3
THE AMINO ACIDS ARE LINKED; THE "EMPTY" tRNA IS DISCARDED; AND SO THE RIBOSOME MOVES ALONG THE MESSAGE, PILING UP AMINO ACIDS, WHICH FOLD THEMSELVES INTO A PROTEIN.
?
Met
AA2
AA3
AA4

D.2

Name ______________________________

Date ____________________ Hour __________

QUESTIONS ON "HOW DNA CODES FOR PROTEINS"

AFTER YOU HAVE read about protein synthesis in your excerpt from the *Cartoon Guide to Genetics,* answer the following questions.

1. What is the relationship between genes and proteins? ____________________

   ____

2. How does RNA differ from DNA? ____________________

   ____

3. What is the molecule that carries the information from a gene to the place where a protein will be made? ____________________

4. What is the process by which such a molecule is made? ____________________

5. What is the enzyme that mediates the process named above? ____________________

6. What is the structure on which proteins are made? ____________________

7. How many bases form one "word" of the RNA message? ____________________

8. What is the technical name for such a group of bases found on mRNA? __________

9. What is another term for protein synthesis? ____________________

10. What is the group of molecules that translates the genetic code? ____________________

11. What is an anticodon? ____________________

12. At the tail end of each tRNA molecule, an ____________ attaches the appropriate ____________________ molecule to the tRNA.

13. What happens when two tRNAs are side by side on a ribosome? ____________________

    ____

14. The first codon on an mRNA always is ____________________.

15. This codes for the amino acid called ____________________.

D.3

The Gene Expression Dance

AS YOU HAVE READ in your excerpt from the *Cartoon Guide to Genetics,* three kinds of RNA molecules cooperate to convert the coded information in DNA (a gene) into a protein with a particular sequence of amino acids.

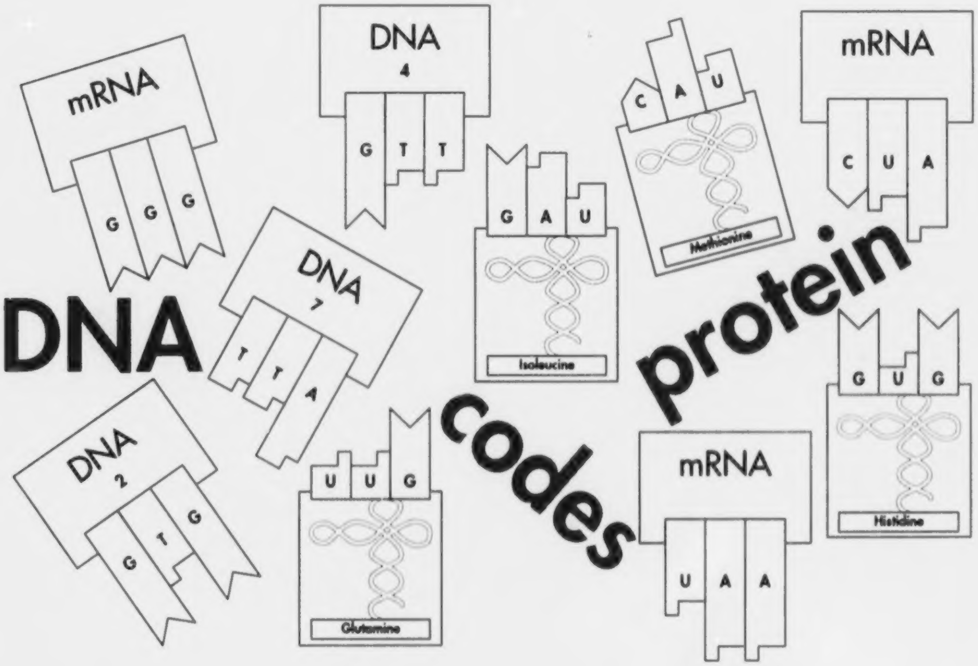
The process in which the nucleotide sequence of a gene (DNA) is used to specify the amino acid sequence of a protein is called **gene expression,** and it consists of two major phases: 1) **transcription**, in which a messenger RNA (mRNA) molecule that is complementary in nucleotide sequence to the gene is synthesized, and 2) **translation**, in which the message carried by that mRNA molecule is used to synthesize the corresponding protein. In this exercise, your class will perform a simulation of these two processes.

MATERIALS

Your teacher will hand out the gene-expression flash cards that you will use in this dance.

PROCEDURE

Your teacher will instruct you how to perform the dance.


mRNA
G G G
DNA 4
G T T
G A U
Isoleucine
C A U
Methionine
mRNA
C U A
DNA 7
T T A
DNA
protein
codes
DNA 2
G T G
U U G
Glutamine
mRNA
U A A
G U G
Histidine

D.4

Name ______________________________

Date ____________________ Hour __________

Paper Proteins: Models for Simulating Gene Expression

MATERIALS

For each student or pair of students:
1 set of paper-protein puzzle pieces

PROCEDURE

Follow the directions below to model the processes of transcription and translation, and to make a paper protein.

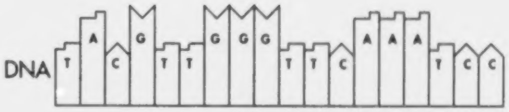

DNA
T A C G T T G G G T T C A A A T C C

Figure 1

1. Place the DNA strip on the desk so that the letters read properly for you (fig. 1).
2. Working from left to right, find the mRNA pieces that match the DNA and line them up (fig. 2). What is the process in which an mRNA molecule that is complementary to a DNA molecule is produced?

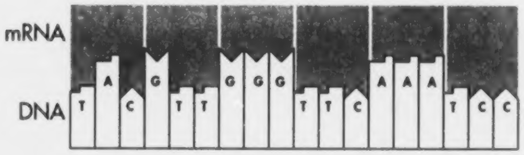

mRNA
DNA
T A C G T T G G G T T C A A A T C C

Figure 2

____

(Answer question 2 here)

3. Separate the mRNA from the DNA to simulate the mRNA moving out of the nucleus to a ribosome in the cytoplasm of the cell. Leave the mRNA pieces lined up next to one another(fig. 3).

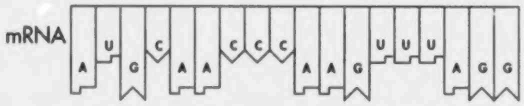

mRNA
A U G C A A C C C A A G U U U A G G

Figure 3

4. Match each of the tRNA pieces to the amino acid piece that fits with it (fig. 4). Lay them out so that all of them are visible.

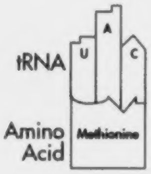

tRNA
U A C
Amino Acid
Methionine

Figure 4

D.4

Name ______________________________

Date ____________________ Hour __________

5. Search for the tRNA that will base pair with the first codon of the mRNA (the one on the left-hand end). Move the tRNA with its attached amino acid into place in the mRNA (fig. 5). Continue with the second codon, and so forth. What is the name for this process in which a protein is produced that has an amino acid sequence specified by an mRNA molecule?

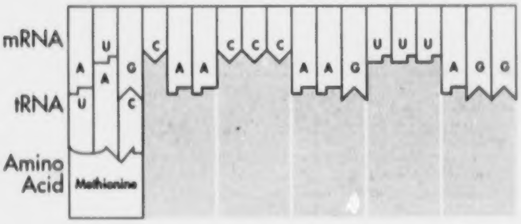


Figure 5

____

(Answer question 5 here)

6. Now pull the string of six amino acids away from the tRNA (fig. 6). These six amino acids represent a new protein. (However, real proteins always contain many more amino acids than this, sometimes more than a thousand.)

Amino Acid
Methionine

Figure 6

After you have completed these steps, use the model pieces to explain the two component processes of gene expression to another student. Then write out the steps in your own words. You may refer to your notes or a book to check for scientific accuracy.

D.5

Name ____________________________

Date ________________ Hour __________

Using the Genetic Code to Translate an mRNA

AT THE HEART OF the regular and predictable relationship between the sequence of nucleotides in any gene and the sequence of amino acids present in the protein for which that gene is said to "code" is a fixed set of nucleotide-to-amino acid relationships that is known as **the genetic code.** Just as the Morse code can be printed in the form of a table indicating which letter of the English alphabet is specified by each combination of dots and dashes, the genetic code is usually printed in the form of a table indicating what kind of amino acid is specified by each possible mRNA codon.

To the right is a DNA coding sequence that codes for part of the hemoglobin molecule. Complete the mRNA strand following base-pairing rules. Then use the mRNA Genetic Code Table on the next page to determine the amino acid sequence for which this piece of a gene codes.

DNA	mRNA	Amino Acid
T A C		
C A G		
G T A		
A A C		
T G T		
G G G		
C T T		
C T C		
T T T		
A G T		
C G C		
C A T		

Congratulations! You have just "synthesized" the first part (12 amino acids) of the protein called B-globin, which is part of the hemoglobin molecules that make your blood red and carry oxygen to cells throughout your body. (Each real B-globin molecule actually consists of a string of 147 amino acids.)

D.5

mRNA GENETIC CODE TABLE

1st base	2nd base				3rd base
	U	C	A	G	
U	UUU = phe	UCU = ser	UAU = tyr	UGU = cys	U
	UUC = phe	UCC = ser	UAC = tyr	UGC = cys	C
	UUA = leu	UCA = ser	UAA = stop	UGA = stop	A
	UUG = leu	UCG = ser	UAG = stop	UGG = trp	G
C	CUU = leu	CCU = pro	CAU = his	CGU = arg	U
	CUC = leu	CCC = pro	CAC = his	CGC = arg	C
	CUA = leu	CCA = pro	CAA = gln	CGA = arg	A
	CUG = leu	CCG = pro	CAG = gln	CGG = arg	G
A	AUU = ile	ACU = thr	AAU = asn	AGU = ser	U
	AUC = ile	ACC = thr	AAC = asn	AGC = ser	C
	AUA = ile	ACA = thr	AAA = lys	AGA = arg	A
	AUG = met	ACG = thr	AAG = lys	AGG = arg	G
G	GUU = val	GCU = ala	GAU = asp	GGU = gly	U
	GUC = val	GCC = ala	GAC = asp	GGC = gly	C
	GUA = val	GCA = ala	GAA = glu	GGA = gly	A
	GUG = val	GCG = ala	GAG = glu	GGG = gly	G

Amino Acid Abbreviations

ala = alanine
arg = arginine
asn = asparagine
asp = aspartic acid

cys = cysteine

gln = glutamine
glu = glutamic acid
gly = glycine

his = histidine

ile = isoleucine

leu = leucine
lys = lysine

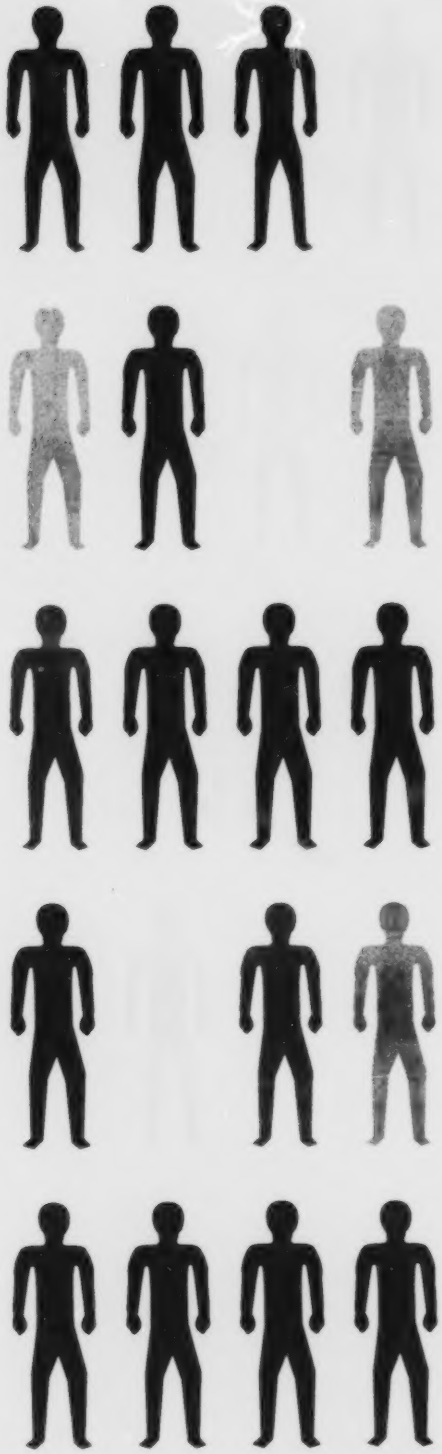
met = methionine

phe = phenylalanine
pro = proline

ser = serine

thr = threonine
trp = tryptophan
tyr = tyrosine

val = valine



CHAPTER 1

DNA: The Hereditary Molecule

SECTION E

How Does DNA Determine a Trait?

E.1

An Introduction to the Connections Between Genes and Visible Traits

BECAUSE EVERY ASPECT OF OUR LIFE depends on proteins, which are encoded by DNA, proteins act as the intermediaries between our DNA and our heritable traits. By now, we are beginning to understand how particular genes determine the amino acid sequences of particular proteins. But how do these proteins determine our various distinctive visible traits?

Most of us have more difficulty understanding this aspect of the gene-to-trait relationship than the DNA-codes-for-protein part. This is not surprising, because most of our obvious traits (such as height, weight, facial features, and skin color) are the results of interactions of many different gene-encoded proteins with one another and with a number of environmental factors. Differences in skin color, for example, are the result of the combined actions of various inherited versions of at least seven different gene-encoded proteins with one another and with the amount of sunshine to which the individual has been exposed. The range of differences that exist in the curliness and color of human hair have an even more complex basis.

To simplify things, the gene-to-trait relationship can be thought of as occurring in steps like this:

- Our genes (which are DNA) specify the structure of our proteins.
- Our proteins, interacting with one another and various environmental factors, determine the chemical and physical properties of each of our cells.
- The combined chemical and physical properties of all of our cells determine the shape, appearance, and behavior of our body as a whole.

In the exercise that follows, we will perform an experiment that illustrates the first two steps of this DNA-to-trait relationship in a very dramatic way. (The third step is omitted, because the organism we will be using for the demonstration is a one-celled organism: a bacterium.) The experiment should also reinforce the idea that each visible trait of an organism is usually the result of several gene-encoded proteins working together. This is because the piece of DNA that we will use to make a bacterium glow in the dark carries seven genes that encode seven different proteins. All seven of these proteins must work together to make a bacterium glow in the dark. If any one of the proteins fails to do its job, no light is generated.

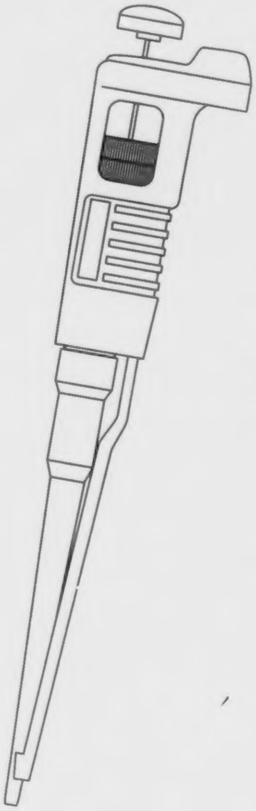
E.2.a

Name ______________________________

Date ____________________ Hour __________

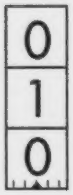
Shine On!

A. How to Read a Micropipettor (1000 µl size)

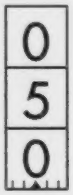


IN MODERN BIOLOGY LABS, it is often necessary to measure extremely small volumes of liquid. The measurements are usually in microliters (µl). One microliter is 1/1,000,000 of a liter. Scientists use instruments called micropipettors to measure these small volumes. The first step in learning to use a micropipettor is to learn how to set it for the exact volume you want to transfer. To do this, you need to look at the window on the micropipettor. The numbers in the window represent the volume of liquid, shown in µl (microliters), that will be measured. The window looks like the ones pictured below. Due to differences in models, your micropipettor may be different than the one pictured.

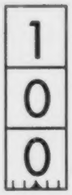
The numbers in the window will look like these when the micropipettor is set to measure the volumes shown in microliters.



100 µl



500 µl



1000 µl

In the windows below, write the numbers that would indicate that the micropipettor was set properly to measure the given volumes.

150 µl

980 µl

240 µl

556 µl

Now that you have filled in the windows, practice setting a micropipettor to the above volumes. Let your teacher check your work, especially when you set the volume of 556 µl. This is a little tricky.

E.2.a

Micropipetting Practice

MATERIALS

For each group of four students:
1 1000 µl micropipettor
2 sterile large tips
1 marking pen
1 tube of colored water (food coloring)
3 pieces of filter paper or white paper towel
1 container for waste disposal

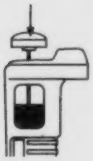


Figure 1

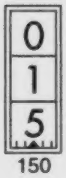


Figure 2

PROCEDURE

1. Check the size of your micropipettor. It should say either 100-1000 or P1000 on the circle on top of the plunger (fig.1).

2. Set the numbers in the window to read 015 (fig. 2). This represents 150 microliters. Remember that a microliter is 1/1,000,000 of a liter. Write 015 and your name on the filter paper.

3. Next, carefully place a tip firmly on the end of the micropipettor (fig. 3). Do not touch the pointed end of the tip with your fingers. This is to prevent your samples from becoming contaminated.

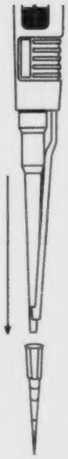


Figure 3

4. To take up a volume of liquid (fig. 4):

   a. Depress the plunger to the first stop. You can feel a resistance. Do not push the button down as hard as you can.

   b. Hold the tube and the micropipettor at eye level. Put just the point of the tip into the liquid found in the tube labeled "CW" for colored water.

   c. Slowly release the plunger button and suction up liquid.

   d. Look at the tip to check for bubbles. If you have bubbles, dispense the liquid back into the tube. You will need to start over.

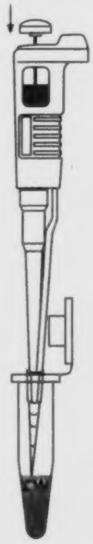


Figure 4

E.2.a

Name ____________________

Date ____________ Hour ________

5. To expel the volume of liquid onto a piece of filter paper (fig. 5).

   a. Hold the tip of micropipettor directly above the labeled filter paper.

   b. Slowly push the plunger button all the way down (past the first stop to the second stop). Look at the tip to make sure all of the liquid came out.

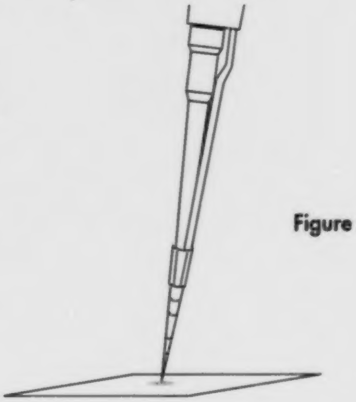


Figure 5

6. To eject the tip:

   a. Hold the tip over a waste disposal container.

   b. Push the eject button.

7. Reset the numbers in the window to read 020 (fig. 6). Write 020 and your name on another piece of filter paper. Follow steps 3-5 to transfer this volume of liquid to the filter paper. What volume in µl does 020 represent?

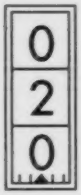


Figure 6

____

(Answer question 7 here)

8. Now reset the numbers in the window to read 024 (fig. 7). Write 024 and your name on another piece of filter paper and follow steps 3-6. What volume in µl does 024 represent?

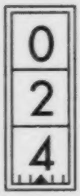


Figure 7

____

(Answer question 8 here)

E.2.b

B. Practicing Microbiological Techniques

IN THIS LABORATORY, we will be using the bacteria *E. coli*. To avoid contamination of your experiment with unwanted bacteria, it is necessary to use sterile techniques and work with all reagents carefully. Wipe your work area with bleach before and after your laboratory and dispose of used pipette tips, tubes, etc. in the containers provided by your teacher. Always wash your hands thoroughly after each laboratory exercise.

MATERIALS

For each group of four students:

- 4 pairs of safety goggles
- 1 petri dish with *E. coli*
- 2 inoculating loops
- 1 tube of sterile nutrient broth (labeled "NB")
- 1 waste container with bleach
- 1 small beaker of 70% alcohol
- 1 petri dish with nutrient agar
- 1 1000 µl micropipettor
- 1 sterile pipette tip
- 1 piece of plastic wrap
- 1 spray bottle of disinfectant
- 1 marking pen

PROCEDURE

1. Put on your safety goggles. Observe your plate of bacteria. To avoid contamination, do not open the lid yet. Notice that the bacteria grow in clumps called **colonies** (fig. 1). Each colony started as one bacterium that then multiplied many times. All of the bacteria in a colony usually are identical. Bacteria grow and divide quite rapidly. Under the best conditions, *E. coli* cells can divide every 20 minutes or so.

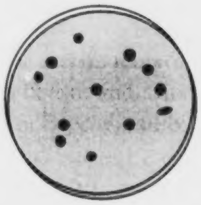


Figure 1

2. Transfer a colony of bacteria into the tube of nutrient broth. Follow these steps:

   a. Carefully remove an inoculating loop (fig. 2) from the package. Do not touch the loop to anything.

   b. Carefully tilt the lid of the petri dish containing the bacteria, without moving the lid away from the dish (fig. 3). This protects the agar from contamination with organisms floating in the air.

Figure 2

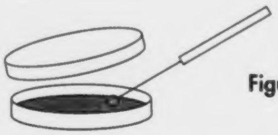


Figure 3

c. Insert the loop end of your inoculating loop and touch a single colony of the bacteria with the tip (fig. 3). Withdraw the loop and replace the lid.

d. Place the loop containing the bacteria into the tube of nutrient broth. Twist the loop to mix the *E. coli* with the broth (fig. 4). Check to be certain the bacteria have come off the loop. Remove the loop. Recap the tube. Dispose of the loop in the bleach solution provided at your lab station.

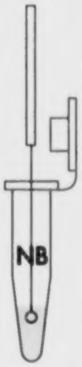


Figure 4

3. Next, you will practice transferring a bacterial suspension from a tube to a petri dish.

a. Label the bottom of a petri dish containing sterile nutrient agar with your name and the date.

b. Set the micropipettor to 100 μl. Using a sterile large tip, transfer from the tube 100 μl of the bacterial suspension that you just made onto the agar in the petri dish (fig. 5). Dispose of the tip in the waste container.

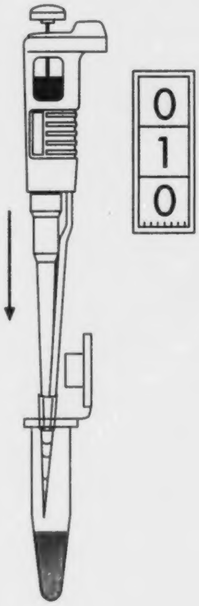


Figure 5

c. Using a new sterile loop, spread the broth containing the bacteria over the surface of the agar. Make a zigzag motion across the agar (fig. 6). Turn the plate 90° and repeat the zigzag motion. Dispose of the loop in the waste container. Put the cover on the plate and seal it with a piece of plastic wrap.

d. Return your plate to your teacher.

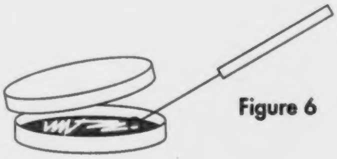


Figure 6

C. Part One

Shine On! Engineering Glow-in-the-Dark Bacteria

MATERIALS

For each group of four students:

4 pairs of safety goggles
1 spray bottle of disinfectant
1 cup with ice
1 tube of sterile water (labeled "W")
1 marking pen
1 tube of plasmid DNA (labeled "DNA")
1 tube of $CaCl_2$ (Labeled "C")
1 petri dish with *E. coli*
2 sterile inoculating loops
1 1000 µl micropipettor
7 sterile pipette tips
1 waste container with bleach
1 small piece of Styrofoam with 2-4 holes
1 tube of nutrient broth (labeled "NB")
1 petri dish with nutrient agar (labeled "WATER")
1 petri dish with ampicillin/nutrient agar (labeled "DNA")
1 roll of plastic wrap

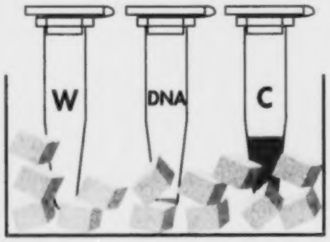
Per class:

1 water bath at 42° C

PROCEDURE

1 Put on your safety goggles. Spray and wipe your work area with disinfectant.

2. Get a cup with ice in it. Locate the tube labeled "W." This tube contains a very small amount of water. Put your initials on the tube. Gently tap the tube on the counter to shake the drop of water to the bottom of the tube. This tube will be your control tube. Place it in your cup of ice (fig. 1).



3. Locate the tube labeled "DNA." This tube contains the DNA with the genes that will cause your bacteria to glow in the dark. Put your initials on the tube. Place it in your cup of ice (fig. 1).

4. Locate the tube labeled "C." It contain a chemical called calcium chloride, $CaCl_2$. Calcium chloride makes bacteria "leaky," so that big molecules like DNA can get inside the cells. Put your initials on the tube and place it in the ice (fig. 1).

5. Locate the petri dish culture of *E. coli*. (You may be sharing this with one or more groups.)

6. Use the inoculating loop to transfer a mass of bacteria to the tube labeled "C." Then use the micropipettor to mix the bacteria with the liquid in tube "C." To do this:

   a. Tilt the lid of the petri dish, holding it over the dish.

   b. Pick a colony off the surface of the agar with the loop, then close the dish (fig. 2).

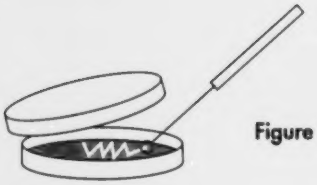


Figure 2

   c. Open tube "C," put the loop with bacteria into the liquid, and gently spin the handle of the inoculating loop to knock off the clump of bacteria (fig. 3). Be sure the bacteria came off the loop! Dispose of the loop.

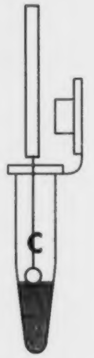


Figure 3

   d. Set your micropipettor to 250 µl.

   e. Place a sterile tip on the micropipettor.

   f. Pick up the tube that now holds the bacteria and gently pipette the fluid in and out to break up the clump of bacteria (fig. 4). Cap the tube and dispose of the tip.

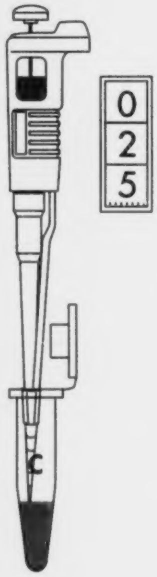


Figure 4

   g. Put the tube with the bacterial mixture into the ice (fig. 5).

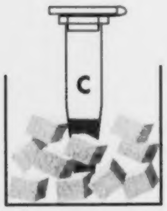


Figure 5

7. Add 250 µl of the bacterial suspension you just made to the tube containing water. To do this:

   a. Set your micropipettor to 250 µl.

   b. Place a sterile tip on it.

   c. Transfer 250 µl of the bacterial suspension from tube "C" to tube "W." Pipette in and out several times to mix (fig. 6).

   d Place tube "W" on ice.

   e. Dispose of the tip.

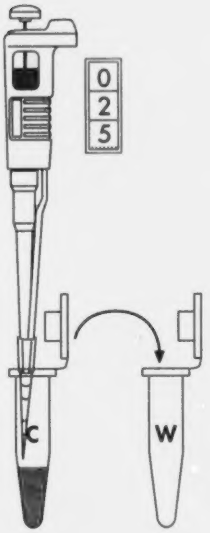


Figure 6

8. Repeat the steps in 7 to add 250 µl of the fluid in tube "C" to tube "DNA" (fig. 7).

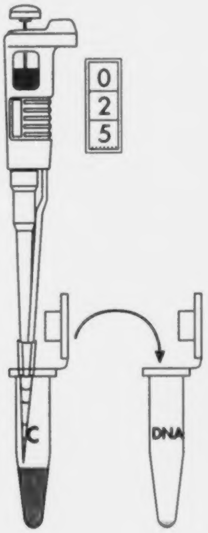


Figure 7

9. Start timing both tubes ("W" and "DNA") at this point. Leave them on ice for 15 minutes (fig. 8).

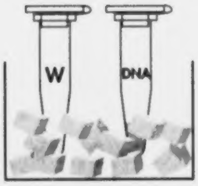


Figure 8

10. At the end of 15 minutes, bring the tubes (still on ice) to the water bath. Check the temperature in the water bath to make sure it is 42°C. Transfer the two tubes from the ice to the water bath (fig. 9). Leave the two tubes in the water bath for exactly 90 seconds.

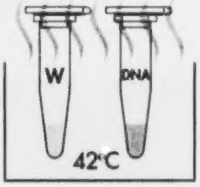


Figure 9

11. Remove the tubes from the water bath and put them back on ice for one minute (fig. 10). After that, they can be at room temperature.

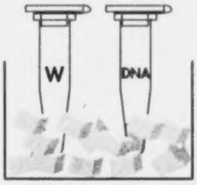


Figure 10

12. Set the micropipettor for 250 µl. Place a new tip on the micropipettor. Locate the tube marked "NB." This tube contains nutrient broth and provides nutrients for the bacteria. Add 250 µl from the tube marked "NB" to each of the two tubes (tube "W" and tube "DNA") (fig. 11). Dispose of the tip.

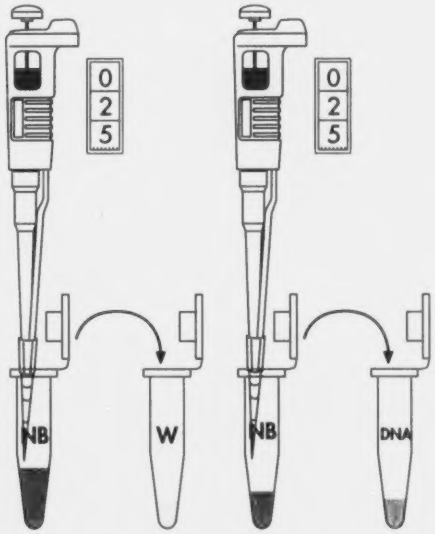


Figure 11

13. You can stop here and store your tubes in the refrigerator until tomorrow, or you can go on to Part Two. Your teacher will direct you.

C. Part Two
Shine On! Engineering Glow-in-the-Dark Bacteria

1. If you stopped at the end of Part One, obtain your "W" and "DNA" tubes from your teacher. If you did not stop, simply continue.

2. Gently shake or invert each tube (fig. 1).

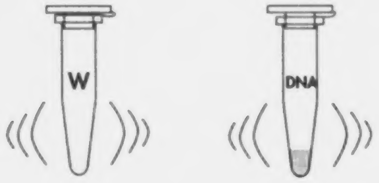


Figure 1

3. Locate the two petri dishes, one labeled "WATER," the other labeled "DNA." Put your group name and hour on the bottom of each dish.

4. Set the micropipettor at 200 µl. Place a new tip on the micropipettor and transfer 200 µl from tube "W" onto the agar in the plate dish marked "WATER" (fig. 2). Dispose of the tip in the waste container.

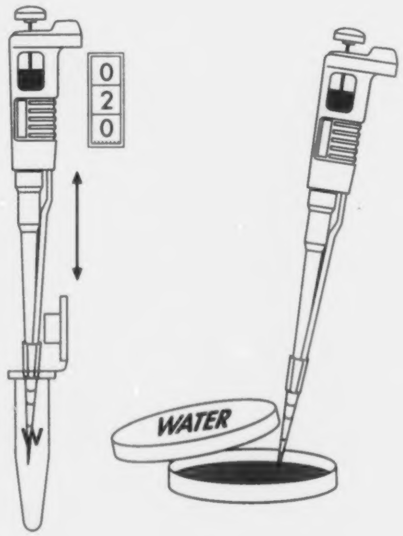


Figure 2

5. Using a sterile loop, spread the bacteria across the surface of the agar. Remember to zigzag, then rotate the dish and repeat (fig. 3). Dispose of the loop.

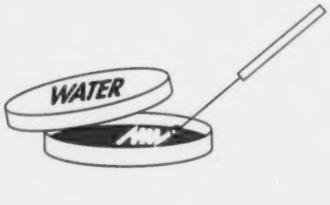


Figure 3

6. Place a new tip on the micropipettor and transfer 200 µl from tube DNA onto the plate marked DNA (fig. 4).

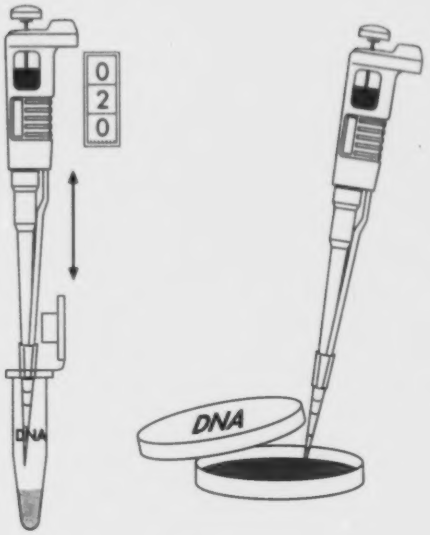


Figure 4

7. Using a sterile loop, spread the bacteria (fig. 5) across the surface of the agar. Remember to zigzag, then rotate the dish and repeat. Dispose of the loop. Seal both dishes with plastic wrap and place them where your teacher directs. Incubate at room temperature.

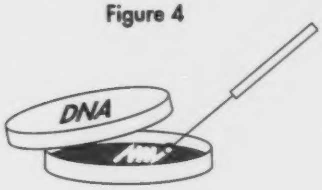


Figure 5

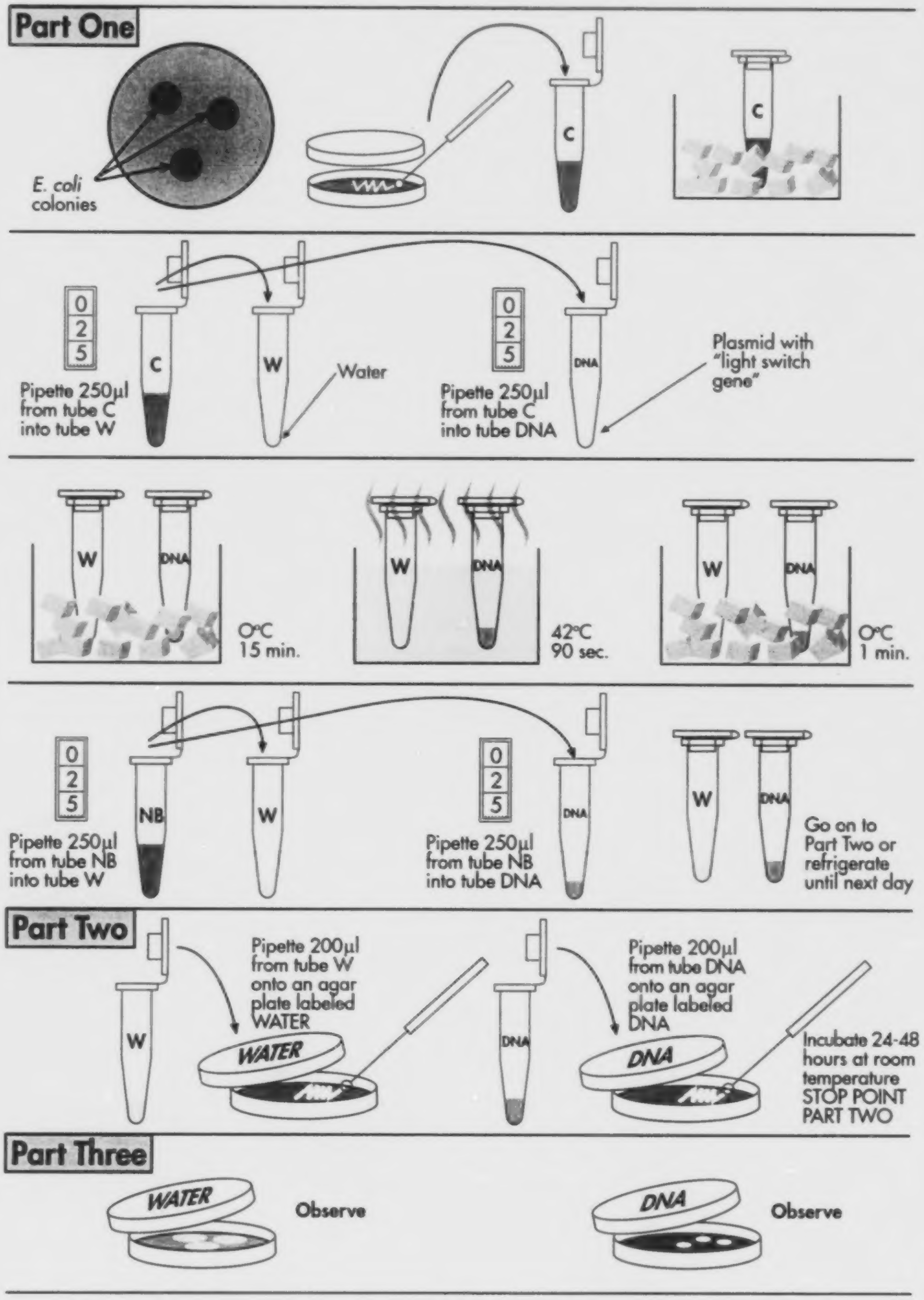
E.2.c

SHINE ON! DIAGRAM OF THE EXPERIMENT

Part One

Part Two

Part Three


Part One
E. coli colonies
C
C
C
0
2
5
C
W
Water
0
2
5
DNA
W
DNA
W
DNA
W
DNA
0
2
5
NB
W
0
2
5
DNA
W
DNA
Part Two
W
WATER
DNA
DNA
Part Three
WATER
DNA

Pipette 250µl from tube C into tube W

Pipette 250µl from tube C into tube DNA

Plasmid with "light switch gene"

0°C 15 min.

42°C 90 sec.

0°C 1 min.

Pipette 250µl from tube NB into tube W

Pipette 250µl from tube NB into tube DNA

Go on to Part Two or refrigerate until next day

Pipette 200µl from tube W onto an agar plate labeled WATER

Pipette 200µl from tube DNA onto an agar plate labeled DNA

Incubate 24-48 hours at room temperature STOP POINT PART TWO

Observe

Observe

E.2.c

Name ______________________________

Date ____________________ Hour __________

C. Part Three
Shine On! Viewing Plates

IT MAY TAKE SEVERAL days before the bacteria that you have genetically engineered begin to glow in the dark. Observe both of your agar plates each day and record what you see on the lines below. Be sure to record when bacterial colonies can first be seen on each plate. Meanwhile, begin filling out the Laboratory Write Up on the next two pages.

Once colonies have appeared on your "DNA" plate, observe both plates each day in a very dark room to see if they have begun to glow. Record the day that this happens. Continue observing the plates daily, and record whether the bacteria are still glowing. When your bacteria have stopped glowing in the dark, ask your teacher for instructions about how to dispose of your plates. Then complete your Laboratory Write Up.

E.2.c

Name ______________________________

Date __________________ Hour __________

ALTERNATIVE ASSIGNMENT: NEWS ARTICLE

YOU ARE TO WRITE an article that is suitable to be printed in a newspaper and describes the Shine On experiment. Make your article interesting, informative, and scientifically accurate. Include the overall idea of the experiment as well as a brief description of the protocol and a conclusion. Your grammar and spelling need to be correct. The best articles may be submitted to the school newspaper.

NEWS ARTICLE GRADING SHEET

____ Scientific accuracy

____ Complete: Included introduction, brief procedure, description of the plates, conclusion

____ Creativity/Interest

____ Grammar/Spelling

____ Total

CHAPTER 2

Passing Traits from One Generation to the Next

CHAPTER 2

Passing Traits from One Generation to the Next

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CHAPTER 2

Passing Traits from One Generation to the Next

SECTION A

What is Inheritance?

A.1

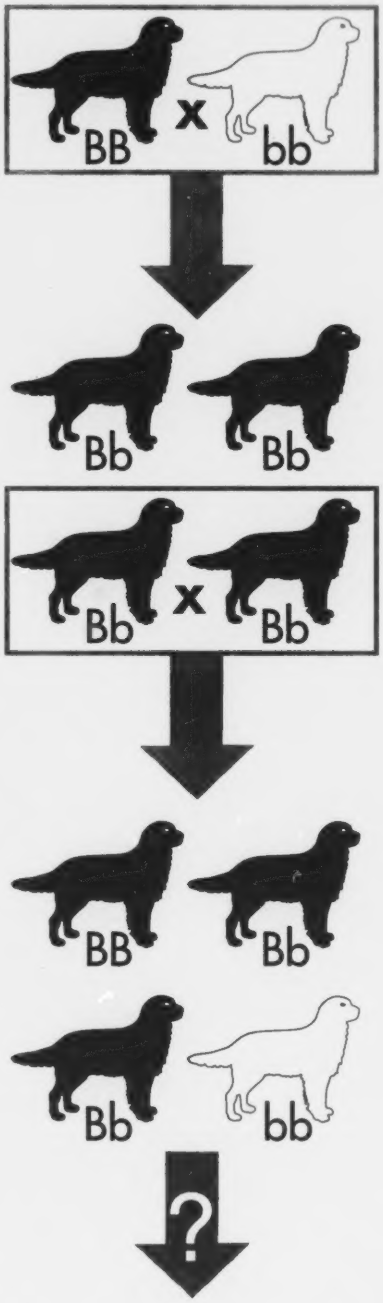
An Introduction to Inheritance

YOU INHERIT YOUR TRAITS, or characteristics, from your parents, and your parents inherited their traits from their parents. Each of us is a unique blend of traits that have been passed on from one generation to the next.

Very simple organisms like bacteria usually pass on a complete and exact copy of their own DNA to their offspring. As a result, their offspring are usually indistinguishable from themselves. Fortunately, humans don't do this, or we'd all be extremely similar to each other in every way.

Humans and other animals have a life cycle that involves the production of specialized egg and sperm cells that must be combined to form a member of the next generation. The nucleus of each egg and each sperm cell contains a copy of just one-half of the DNA of the adult who produced it. When the sperm and egg nuclei fuse after the egg has been fertilized, a new nucleus is produced that contains a copy of one half of the mother's DNA and one half of the father's DNA. It is this combination of DNA molecules, in which genes from the mother and father are now intermingled, that will direct the development of a new individual.

Although everyone inherits half of their DNA from their mother and half from their father, no one ever turns out to be exactly halfway between their two parents in their heritable features. In some of your traits you may resemble your mother, while in others you may resemble your father. And in some of your traits you may resemble one of your grandparents more than either of your parents. When we were growing up, some of us may have heard statements like "He has his mother's eyes and his father's nose, but he sure inherited Grandpa Bill's big ears!"



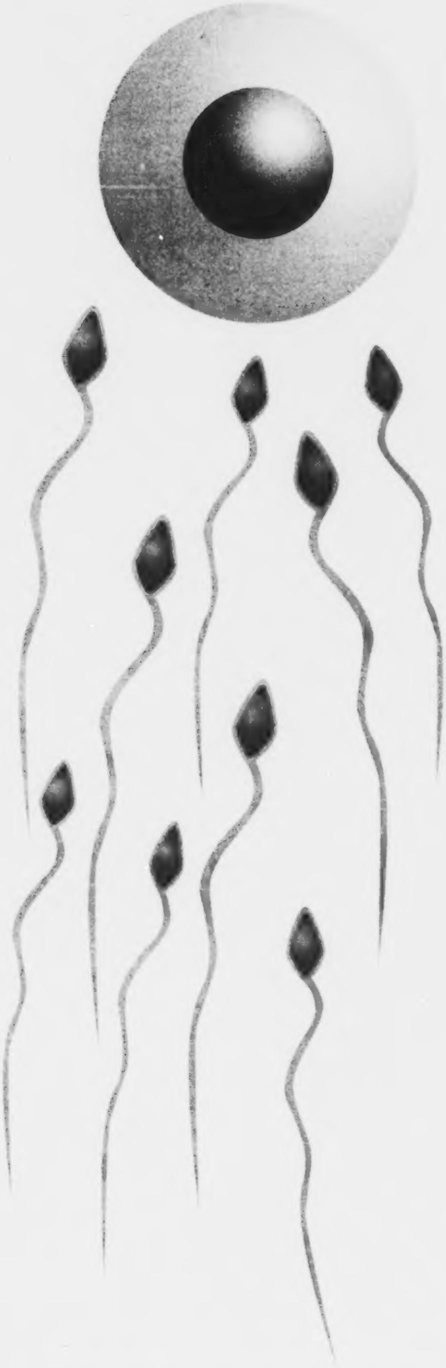
A.1

The reason that each of you develop your own unique mixture of family traits is that the pair of genes for each trait that you inherit from your parents often do not have equal effects on your development. For example, as your hair follicles were developing, the gene for curly hair that you got from your mother may have been **dominant** over the gene for straight hair that you got from your father. In this case your hair will be curly, like your mother's. In the development of some other body parts, however, the reverse may have been true, and your father's genes may have been dominant over those of your mother. Genes that did not reveal their presence during development of your visible traits are said to be **recessive** to their dominant counterparts. Recessive genes are passed on from generation to generation just like dominant genes, but they only reveal their presence in individuals that did not happen to inherit a copy of a dominant gene for that trait.

The dogs in the diagram on the opposite page can be used to illustrate this sort of dominant-recessive relationship between two versions of a single gene. The top part of the diagram indicates that when a particular black and white dog mated, all of their offspring were black. However, the middle part of the diagram indicates that when two of those black dogs mated, about 1/4 of their puppies were white! We can account for this inheritance pattern by assuming that every dog inherits one copy of the gene for coat color from each of its parents, and that this coat-color gene comes in two forms: a ***B*** form that causes black hair, and a ***b*** form that sometimes causes the hair to be white. The reason that the ***B*** form of this gene is said to be dominant is because dogs having only one copy of it (dogs symbolized ***Bb*** in the diagram) are just as black as dogs having two copies of it (dogs symbolized as ***BB***). In contrast, the ***b*** version of the gene is said to be recessive, because it only has an effect on coat color in dogs that lack a ***B*** gene (such as the ***bb*** dogs at the top and bottom right).

What sort of difference between two different forms of a gene causes one to be dominant and the other to be recessive, you ask? GREAT QUESTION! For the answer we need to refer back to things we learned in Chapter 1. Recall that the function of most genes is to specify the amino acid sequence of a particular protein, and that many of these proteins act as enzymes that mediate particular chemical reactions. The genes involved in determining the coat color of the dogs in our diagram encode alternative forms of an enzyme that is required to make "melanin," the pigment that is present in black hair. Whereas the ***B*** gene encodes an active form of this enzyme, the ***b*** version of the same gene encodes a damaged, inactive form of the enzyme. The reason that the ***B*** gene is dominant is that one copy of ***B*** is all that is necessary to make enough enzyme – and thus enough melanin – to turn the hair black. Since one copy of ***B*** is enough to make the hair black, the ***b*** version of the gene is recessive, because it can only exert an effect on hair color when the active form of the enzyme encoded by the ***B*** version of the gene is absent. Many dominant-recessive relationships between gene pairs have a similar basis.

In this chapter, you will use a variety of organisms – real and imaginary – to study (a) the patterns in which genes are sorted out during the formation of egg cells and sperm cells, (b) now those genes recombine when sperm and egg fuse, and (c) how they then determine the traits of the offspring. Before doing that, however, we will take a minute to consider how biologists who are interested in studying heredity decide which organisms to use for their studies.



CHAPTER 2

Passing Traits from One Generation to the Next

SECTION B

How Does a New Generation Get Started?

B.1

Model Systems for Studying Heredity and Development

THE DNA PRESENT IN a human egg and sperm cell at the moment that they fuse contains enormous potential: the potential to direct formation of the most complex form of life the world has ever known – a human being. But the potential residing in DNA is of little significance until it becomes transformed into a physical reality. This happens through a long, slow process known as **development**. Human development begins as soon as egg and sperm fuse, while the individual is still a tiny one-cell **zygote**, and continues long after an **embryo** (a developing, unborn individual) has been transformed into a squawking newborn baby.

By now we know that genes code for proteins. But how do the proteins encoded by human DNA direct the transformation of a fertilized egg into an adult with blood and guts, muscles, and nerves, all in the right places, properly connected, and working together? And in the process, how do particular versions of certain genes that have been passed on in an egg or sperm give rise to the heritable traits that distinguish one human being from another?

Although no one can give completely satisfying answers to all such questions, enormous progress towards answering them has been made recently. Indeed, more has been learned about the twin processes of human heredity and human development during the period of time in which you have been alive than in all the prior centuries of human history combined.

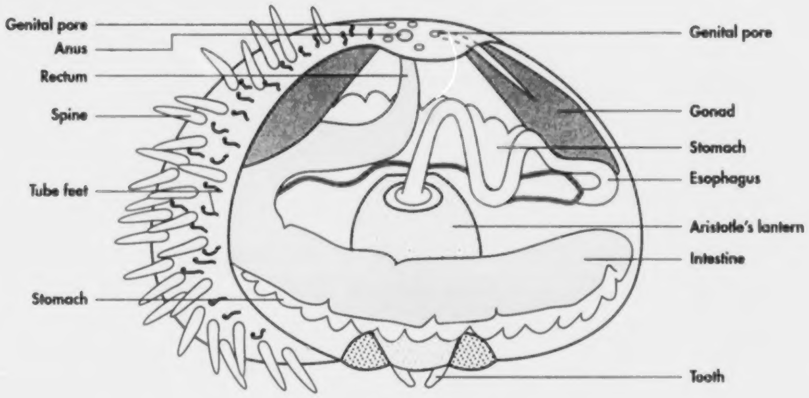
Very little of this new knowledge has come from studying human embryos themselves. Detailed studies of human embryos are impractical for both technical and ethical reasons. Therefore, most of what we now know about how human genes control human development has been derived by studying simpler organisms, such as yeast, fruit flies, sea urchins, frogs, and mice. Such organisms are frequently called **model systems**, because each of them has features that makes it the most suitable organism for studying certain particular aspects of heredity and/or development. But because none of them is equally suitable for studying all interesting aspects of heredity and development, they continue to be studied in parallel.

The most astonishing thing about such studies of model systems is the extent to which they provide information that is applicable to human beings. For example, as soon as it was discovered that one particular gene is essential for initiating eye development in fruit flies, it was quickly shown that the human version of that gene plays a very similar role in the development of our eyes. Moreover, certain serious abnormalities in human eye development and function have now been traced to defects in the DNA sequence of that very gene. Had it not been for the earlier studies of eye development in flies, however, we might never have discovered the important role of this gene in human eye development.

We will encounter several model systems, including some that are mentioned above, in the next few exercises. As we do, the qualities that make each of them useful for particular kinds of studies of heredity and development will be outlined.

B.2

Starting a New Generation: Sea Urchin Fertilization



A cut-away view of a sea urchin

INTRODUCTION

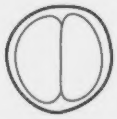
Sea urchins have long been the favorite model organism for studying **fertilization**, the process by which a sperm fuses with an egg. Fertilization converts the egg into what is called a **zygote** and triggers the changes that will transform it into a developing **embryo**. Sea urchins are favored for such studies for several reasons. First, a single female sea urchin may produce as many as 100,000 eggs at one time. Second, both eggs and sperm are released into the sea water, so fertilization occurs outside the body, where it is easily observed. Third, sea urchin eggs and embryos are translucent and just the right size to be easily studied with a microscope. And fourth, all of the embryos from a batch of fertilized eggs develop on the same schedule. As a result, it is easy to get enough embryos to perform chemical analyses at each stage of early development. Many of the things that have been learned by studying fertilization of sea urchin eggs have been useful in developing methods for *in vitro* fertilization of human eggs, to produce what journalists call "test tube babies."

Sea urchins are members of the phylum Echinodermata (which means "spiny-skinned"); this phylum also includes the starfish and sand dollars. Sea urchins live in shallow near-shore waters in all the oceans of the world. The nearly round shell of an urchin, which is called its **test**, is covered with movable, protective spines. Between the spines are small, muscular **tube feet,** each of which has a suction cup on the end. The urchin uses the tube feet on the bottom of its test to walk about slowly on the ocean floor. But it can also use the ones on the top and sides to right itself if it happens to get flipped on its back by a wave or a potential predator.

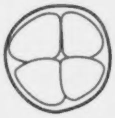
With your teacher's assistance, pick up a sea urchin. The spines will not hurt you. On the top, locate the anus in the center and the genital pores around it. Then locate the mouth on the bottom. Notice the soft ring of skin (the oral membrane) around the five white teeth. The five-sided jaw apparatus, called Aristotle's lantern, is a complicated chewing organ consisting of five jaws and teeth that are adapted for scraping algae off rocks. Sea urchins are well adapted to life on the ocean bottom. They feed by moving on top of their food, holding it down with their spines and tube feet, and then tearing it to bits with their teeth. A sea urchin's diet, in addition to algae, may include coral, sponges, mussels, sand dollars, and kelp.

LIFE CYCLE

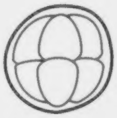
Like other animals, sea urchins pass their DNA on to their offspring in the nuclei of eggs and sperm that fuse during sexual reproduction. As you might have guessed, eggs are produced by female urchins and sperm by males. But to human beings, male and female urchins look identical. So there is no way of knowing which is which until they have been stimulated to **spawn** or release their **gametes,** which depending on their sex, are either eggs or sperm. As a result, your class may have to stimulate several urchins to spawn in order to get gametes of both types. Then you will fertilize some eggs with sperm and watch them as they divide to form multicell embryos.



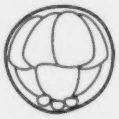
2-cell stage
(~ 1 hour after fertilization)



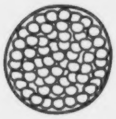
4-cell stage
(~1 1/2 hours)



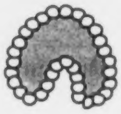
8-cell stage
(~2 hours)



16-cell stage
(cells are unequal in size; ~2 1/2 hours)



Blastula – a hollow ball of cells. Cleavage is over; blastula will break out of fertilization membrane and begin to grow. (~ 6-12 hours)



Gastrula – the ball gets a dimple, which deepens into a tube – the gut. (~ 1 day)

A sea urchin embryo develops into a swimming **larva**, a juvenile stage that bears no resemblance to an adult. In the ocean, the larvae swim about for many weeks or months, feeding on various tiny prey organisms and growing. Then (like caterpillars turning into butterflies) the larvae eventually undergo **metamorphosis** and transform into small adult sea urchins. Because urchin larvae and adults grow rather slowly and require particular kinds of algae and tiny animals as food at each stage of growth, it is extremely difficult to get them to go through a complete life cycle in captivity. Therefore, although sea urchins

B.2

Name ____________________

Date ______________ Hour ________

provide a useful model system for studying fertilization and the genes that are expressed during the early stages of development, they are not very useful for studying other aspects of heredity and development.

MATERIALS

For each group of four students:

- 1 or 2 glass depression slides
- 2 or 3 dropping pipettes
- a compound microscope
- an egg suspension
- a sperm suspension
- a sample of previously fertilized eggs

PROCEDURE

Collecting Sea Urchin Gametes

1. Your teacher will demonstrate the technique used to get the sea urchins to spawn. It involves injecting a bit of a KCl solution into the body cavity. This creates a mild stress that (like many other mild stresses) causes the urchins to release their gametes. The eggs will be collected in a beaker of sea water. The sperm will be collected in a dry tube, and diluted with sea water later.

2. When your teacher informs you that the eggs are ready, use a pipette to pick up a drop of an egg suspension and place it on a clean glass depression slide. Examine the slide in your microscope with a 4X or 10X objective. The eggs are small and round. Draw a picture of their appearance in the microscope.

Sea urchin eggs

3. You will have the best chance of being able to view the fertilization process if you have 10-20 eggs in your depression slide. If you have more than that, remove part of the sample and replace it with sea water.

B.2

Name ____________________

Date ______________ Hour __________

Fertilization

4. Your teacher will inform you when a sperm suspension is ready. Using a clean pipette, add a drop of this sperm suspension to the egg suspension on your slide. It is very important not to add too many sperm. If you do, the water will become too cloudy to see the eggs clearly, and excess sperm will cause abnormal development of the embryos. The correct amount is when 10-100 sperm can be seen around each egg.

5. Observe the sperm-egg mixture with a 4X or 10X objective. Draw what you observe. Fertilization will be evident when a fertilization membrane forms around the egg. Draw what this looks like.

Sperm and egg mixture

Fertilized sea urchin egg

6. The eggs that you fertilized in the depression slide probably will not continue to develop normally while being viewed with the microscope; the light source will heat them up too much. Your teacher will prepare a mixture of sperm and eggs in a beaker and leave it on the bench top. You should examine a drop of this suspension every 15 minutes or so to monitor development. The first division should occur about an hour after fertilization. Draw pictures of the divisions. Your teacher may also have samples of eggs that were fertilized some time before your class began that you can examine to see more advanced stages of development.

1 hour

1 1/2 hours

2 hours

2 1/2 hours

B.2

Name ______________________________

Date ____________________ Hour __________

POSTLAB QUESTIONS

1. If the body cells found in one particular species of an adult sea urchin have 14 chromosomes, how many chromosomes would an egg or sperm of that species have?

   ____

2. What do you think would happen if one of the gametes (either the egg or the sperm) had the wrong number of chromosomes? Why?

   ____

3. What are some differences between a fertilized and unfertilized egg?

   ____

4. What is the function of the fertilization membrane? Why would that be important?

   ____

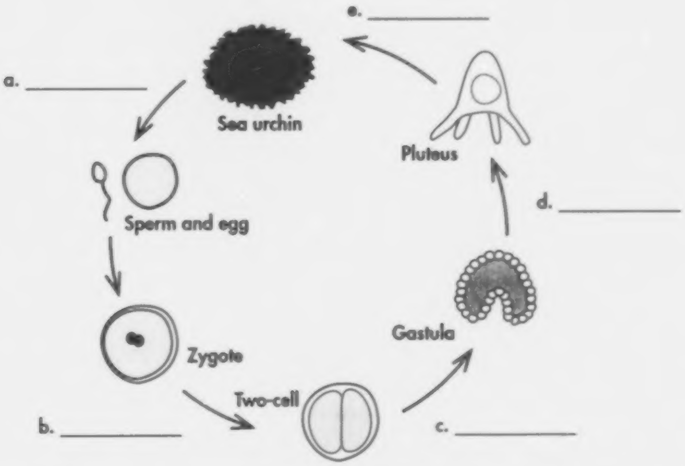
5. What happens to the fertilized egg about an hour after fertilization?

   ____

6. When a cell of an embryo divides, how are each of the newly formed cells similar to one another and to the original fertilized egg but different from the unfertilized egg?

   ____

7. Mitosis and meiosis are essential aspects of the cycle of life and development. Complete the diagram below by writing mitosis or meiosis on the correct lines.



B.3

Name ______________________________

Date ____________________ Hour __________

The Miracle of Life

WITH ANY LUCK, IN the preceding exercise you saw male and female sea urchins release sperm and eggs and then you watched those cells fuse and initiate the development of a new generation. Now, through the wonders of modern technology, you will be able to follow this up with extraordinary views of the equivalent processes in human beings. The exceptional photography in the video *The Miracle of Life* will take you on a journey through the reproductive tracts of both the human female and the human male and will allow you to observe the numerous stages of the human reproductive process- from the early stages of gamete development, through the moment of conception, and to the moment of birth.

Read the questions on the work sheet below and on the next page before the video begins. As you watch the video, take notes to help you answer the questions later. Then write your answers on the work sheet in complete sentences.

THE MIRACLE OF LIFE QUESTIONS

1. Describe the journey of the egg as it becomes mature and travels toward the sperm.

2. Describe the journey of the sperm as they leave their site of origin and travel toward the exterior.

3. About how many sperm does a man produce in his lifetime?

4. About how many sperm are released in a single ejaculation?

5. After sperm are released into the vagina, how long are they viable?

B.3

Name

Date Hour

6. Describe the barriers that the sperm face as they travel up the female reproductive tract toward the egg.

7. Where is the egg when the sperm reach it?

8. About how many sperm reach the egg?

9. What happens to the sperm after it enters the egg?

10. When does the fertilized egg begin dividing?

11. What is the fertilized egg called after it divides?

12. How long after fertilization does the embryo implant itself in the uterine wall?

13. Describe the human embryo at the following stages:
Example: 4 weeks: It has arm buds and the beginnings of eyes.

5 weeks:

6 weeks:

7 weeks:

8 weeks:

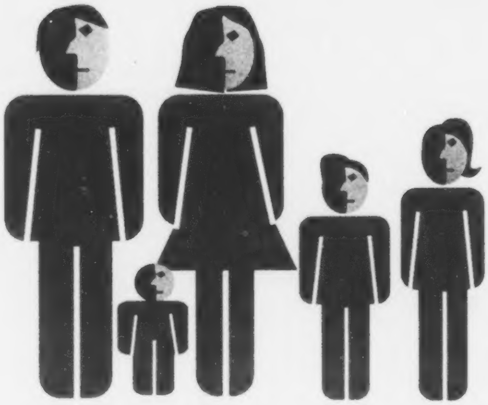
10 weeks:

14 weeks:

18 weeks:

CHAPTER 2

Passing Traits from One Generation to the Next

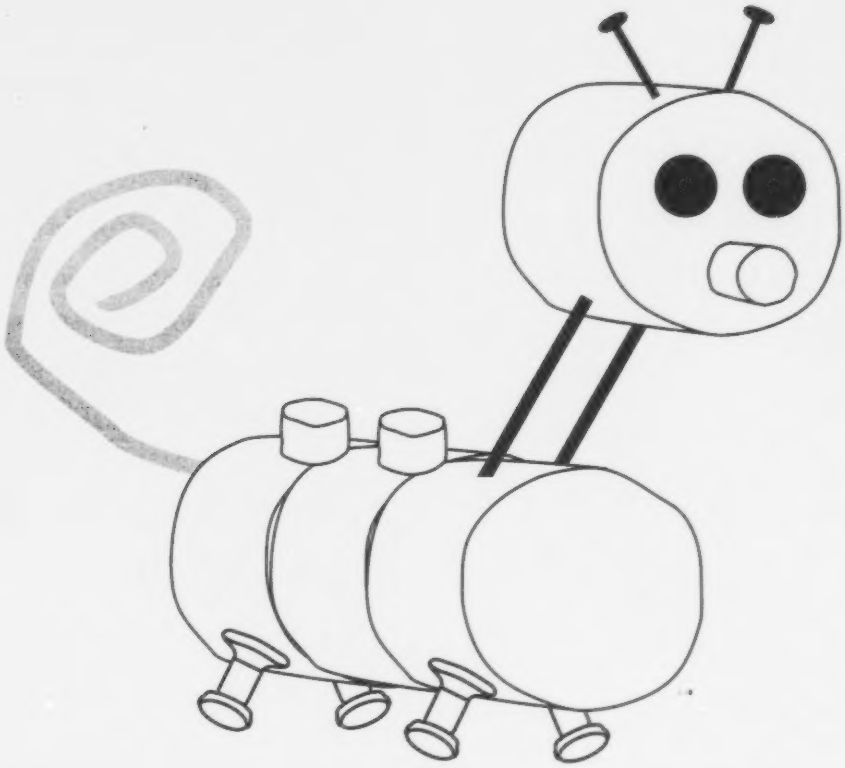


SECTION C

If All the Kids Have Mom and Dad's Genes, Why Don't They All Look Alike?

C.1

Really Relating to Reebops



INTRODUCTION

NOW FOLKS, HERE WE REALLY DO have a model system for studying heredity. (A model system in the same sense that the term "model" was used in Chapter 1.) Reebops are imaginary creatures that were invented by Patti Soderberg at the University of Wisconsin. As you create baby Reebops from marshmallows and other objects, they can help you see how the visible traits of a baby are related to the combination of genes that it inherited from its mom and dad (and why all the kids in the family don't always look alike) Have fun Reebopping!

MATERIALS

An envelope containing one set of red chromosomes and one set of green chromosomes

Boxes at the front of the room containing Reebop body parts, such as marshmallows, toothpicks, nails, etc.

C.1

Name ______________________________

Date ____________________ Hour __________

PROCEDURE

If you find any words in the instructions below that you do not understand, check out the Genetic Glossary on page 88.

1. You and your lab partner will receive an envelope that contains 14 red chromosomes that belong to Mom Reebop and 14 green chromosomes that belong to Dad Reebop. Decide which of you will act as Mom and which will act as Dad. Place your chromosomes on the table in front of you, letter side down. Your lab partner should do the same with the other set of chromosomes.

2. Arrange your 14 chromosomes into pairs by length and width. Select one chromosome from each of your seven pairs and place all seven in a special "gamete" (egg or sperm) pile. Your lab partner should do the same. The leftover chromosomes should now be returned to the envelope.

   What type of cell division has just occurred? ______________________________

3. Combine the seven red and seven green chromosomes from the two gamete piles to form a "baby" pile. Now each Reebop baby will have 14 chromosomes just like Mom and Dad did. But half will be red and half green, indicating that half came from Mom and half from Dad.

4. Line up the chromosomes contributed to the baby by Mom and Dad in pairs of similar size, letter side up. You will see that each chromosome in a pair carries a gene of similar type (same letter of the alphabet).

   Some chromosome pairs might carry the same allele (either both capital letters or both lower case), indicating that the baby is homozygous (has two alleles of the same type) for the kind of gene carried on that chromosome.

   Other chromosome pairs might carry one dominant (capital letter) allele and one recessive (lower-case) allele, indicating that the baby is heterozygous (has two alleles of different type) for the kind of gene carried on that chromosome.

   The combination of genes carried on these seven chromosome pairs defines your Reebop baby's genotype (genetic constitution). Record this genotype on the lines below.

   ___ ___   ___ ___   ___ ___   ___ ___   ___ ___   ___ ___   ___ ___

C.1

Name ________________________________

Date ____________________ Hour ____________

5. Refer to the Reebop Genotype-Phenotype Conversion Table on page S94 to determine your baby's phenotype. Record the phenotype on the lines below, keeping the phenotypic traits in the same order as the genes you listed in step 4.

____________ ____________ ____________

____________ ____________ ____________ ____________

6. You are now ready to construct your Baby Reebop. Collect the body parts that you will need and return to your desk to build your baby.

C.1

GENETIC GLOSSARY

allele: one of two or more forms of a gene that can exist at a single locus.

chromosome: a structure in the nucleus of a eukaryotic cell that contains a linear array of many genes. A chromosome is composed of a single DNA double helix molecule wound around many protein molecules that stabilize it and regulate its function.

codominant: refers to a pair of alleles, both of which exert an effect on the phenotype when they are present together. In codominance, the heterozygote has a phenotype different from that of either homozygote and sometimes (but not always) is intermediate in phenotype.

diploid: having two complete sets of chromosomes, one set derived from the mother and one from the father.

dominant: refers to an allele that has the same effect on the phenotype whether it is present in the homozygous or heterozygous condition. (Thus, if *A* is a dominant allele, individuals with the *AA* and *Aa* genotypes have the same phenotype.)

genotype: the specific combination of alleles that an individual possesses at one or more loci.

haploid: having only one set of chromosomes (as in a sperm or egg nucleus).

heterozygous: having two different alleles at a particular locus.

homozygous: having two identical alleles at a particular locus.

incomplete dominance: a form of codominance in which the heterozygote is about halfway between the two homozygotes in phenotype. (For example, if homozygous plants have red or white flowers and the heterozygous plant has pink flowers, the situation is sometimes called incomplete dominance. But it is just a special type of codominance.)

locus: a region of a chromosome or DNA molecule where a particular kind of gene, coding for a particular kind of protein, is located. Different variants at a single locus are known as alleles.

meiosis: the "reduction division" in which a diploid cell divides to produce haploid cells that will function as gametes (eggs or sperm).

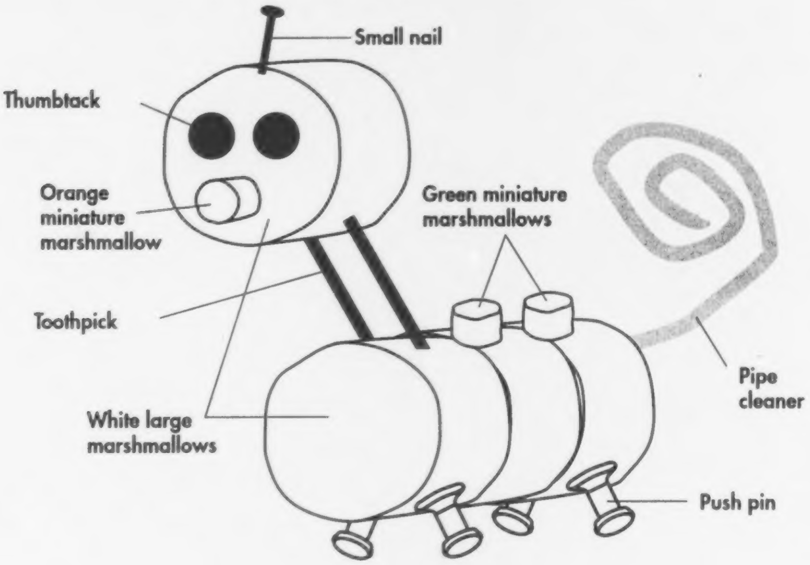
phenotype: the outward appearance of an individual with respect to one or more traits that is associated with some particular genotype.

recessive: refers to an allele that has no effect on the phenotype unless it is present in the homozygous condition.

recombination: the process in which two haploid sets of chromosomes are brought together in a pair of gametes to produce a new diploid offspring. Usually this new diploid will be different in genotype from both of its parents.

C.1

REEBOP GENOTYPE-PHENOTYPE CONVERSION TABLE


Small nail
Thumbtack
Orange miniature marshmallow
Green miniature marshmallows
Toothpick
Pipe cleaner
White large marshmallows
Push pin

GENOTYPE	PHENOTYPE
DD	Three body segments
Dd	Three body segments
dd	Two body segments
AA	Two antennae
Aa	One antenna
aa	No antennae
NN	Red nose
Nn	Orange nose
nn	Yellow nose
EE	Two eyes
Ee	Two eyes
ee	One eye
MM	Three green humps
Mm	Two green humps
mm	One green hump
TT	Curly tail
Tt	Curly tail
tt	Straight tail
LL	Blue legs
Ll	Blue legs
ll	Red legs

Note: Toothpicks function as the bones and ligaments that hold the Reebops together.

C.1

Name ________________________________

Date ____________________ Hour __________

REEBOP REVIEW

1. Define the following terms and give an example of each from this activity. (You may refer to the Genetic Glossary.)

   allele: ________________________________

   genotype: ________________________________

   phenotype: ________________________________

   homozygous: ________________________________

   heterozygous: ________________________________

2. If a Reebop female with a red nose and a Reebop male with a yellow nose marry and have children, what genotype and phenotype for nose color will their children have? (You may refer back to the Reebop Genotype-Phenotype Conversion Table.)

   genotype ____________________ phenotype ____________________

3. If a Reebop female with one antenna and a Reebop male with no antennae marry and have children, what genotypes and phenotypes might their children have with respect to number of antennae?

   genotypes __________ __________ phenotypes __________ __________

4. If a Reebop female with one antenna and a Reebop male with one antenna marry and have children, what is the probability that they will have a baby with no antennae? (If you have a problem with this question, check out section C.2!)

   ____

5. If a Reebop female with two green humps and a Reebop male with two green humps marry and have children, what is the probability that their first baby will have two green humps?

   ____

6. If a Reebop female with three green humps and a Reebop male with three green humps marry and have children, what is the probability that they will have a baby with two green humps?

   ____

7. If a Reebop baby has a straight tail, but both of his parents have curly tails, what are genotypes of the two parents?

   ____

C.1

Name ______________________________

Date ____________________ Hour __________

CLASS REEBOP DATA

FILL IN THE NUMBER of Reebops found in your class with the following heritable traits:

Antennae		**Nose color**		**Humps**	
One	_____	Red	_____	One	_____
Two	_____	Orange	_____	Two	_____
None	_____	Yellow	_____	Three	_____

Eyes		**Segments**		**Tail**		**Leg color**	
One	_____	Two	_____	Curly	_____	Blue	_____
Two	_____	Three	_____	Straight	_____	Red	_____

ANALYSIS OF REEBOP FINDINGS

1. Describe the phenotypes of Mom and Dad Reebop.

   ____

2. Using the information in the Reebop Genotype-Phenotype Conversion Table, list all the possible genotypes that would produce the phenotypes exhibited by Mom and Dad.

   ____

3. How many of the Reebop babies in your class have the same phenotypes as Mom or Dad?

   ____

4. Do any two babies in your class have exactly the same phenotypes?

   ____

5. Why do some Reebop babies have traits that are not seen in either Mom or Dad?

   ____

C.1

Name ____________________

Date ____________ Hour ________

6. Which Reebop traits are dominant?

7. Which Reebop traits exhibit codominance?

8. Use the information you have about the phenotypes of all of the Reebop babies in your class to figure out what the genotypes of Mom and Dad Reebop are. Write the answer below.

9. If you know the genotype of the parents, is it possible to predict all of the possible genotypes of babies that they might produce?

10. If you know the genotype of the parents, is it possible to predict the genotype of any particular baby, such as their first one?

11. The Reebops appear to have only one gene on each chromosome. Do you think this is true of real, living organisms?

C.2

Determining Genetic Probabilities With a Punnett Square

INTRODUCTION

Often someone would like to know how likely it is that two parents with a particular phenotype will have an offspring with the same or a different phenotype. For example, a cat breeder may want to know how likely it is that if a black cat and a white cat mate they will produce a white kitten. Or two people who both have parents with a heritable disease may want to know how likely it is that one of their children would have that same disease.

Many years ago a man by the name of Punnett figured out how to use a square diagram to answer such questions. Biologists have been using this method, a **Punnett square**, for similar purposes ever since. It works for any organisms – plants, animals, or Reebops – that reproduce sexually. There are only two requirements: (1) we must be able to figure out what the genotypes of both parents are with respect to the trait we are interested in, and (2) we must know what phenotype is associated with each possible combination of the alleles that are involved.

PROCEDURE

Here we will use a Punnett square to answer the following question: If a Reebop female with one antenna and a Reebop male with one antenna marry and have children, what is the probability that one of their children will have two antennae? But the following steps would be used for answering all such questions that involve one locus with two alleles.

1. Determine what the genotypes of the parents are with respect to the trait of interest. According to our Genotype-Phenotype Conversion Table in the previous lesson, a Reebop with one antenna has the genotype Aa. So both the female and the male have the genotype Aa.

2. Draw a larger square that contains as many smaller squares in each direction as there are alleles to be considered for each of the parents (fig. 1). In this case, two.

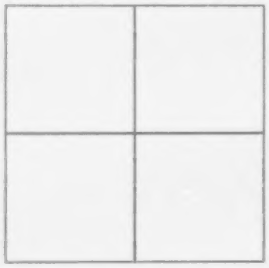


Figure 1

C.2

Name ______________________________

Date ____________________ Hour __________

3. Above the two boxes at the top place letters corresponding to the genotypes of the haploid eggs a female of the specified genotype (in this case, *Aa*) would produce following meiosis (fig. 2).

4. Beside the two boxes on the left place letters corresponding to the genotypes of sperm a male of the specified genotype (*Aa*) would produce following meiosis (fig. 2).

Kinds of eggs produced by an *Aa* female

Kinds of sperm produced by an *Aa* male

	A	a
A		
a		

Figure 2

5. Next, place letters in each of the smaller boxes indicating the genotype that would be produced if an egg of the type indicated above were combined with a sperm of the type indicated to the left (fig. 3). These are the four genotypes that would be formed with equal likelihood when an egg is selected at random and combined with a sperm that is also selected at random.

Kinds of eggs produced by an *Aa* female

Kinds of sperm produced by an *Aa* male

	A	a
A	*AA*	*Aa*
a	*aA*	*aa*

Figure 3

6. Now in each box place words indicating the phenotype that is associated with the genotype specified in that box (fig. 4).

Kinds of eggs produced by an *Aa* female

Kinds of sperm produced by an *Aa* male

	A	a
A	*AA* (2 antennae)	*Aa* (1 antenna)
a	*aA* (1 antenna)	*aa* (0 antenna)

Figure 4

7. Now determine how many of the four equally likely genotypes will result in the phenotype that the question asked about. In this case we get the answer that the probability of having a baby with two antennae is 1 in 4.

C.3

Exploring Human Traits: Create-a-Baby

INTRODUCTION

Our imaginary friends, the Reebops, helped us understand how it is possible for two parents who look very similar to one another to have a family of children that all look different from their parents and from one another. The secret lies in the processes of meiosis and recombination. These two processes work in sequence, first to select various parental alleles at random and then to bring them together to form combinations that are entirely new and different.

In this exercise, you and a partner will simulate human meiosis and recombination to produce an imaginary baby that will have some features that resemble your features and some that do not. In the process, you will learn that heredity is often a bit more complicated than in the simple examples we encountered with the Reebops, in which each trait was controlled by a single pair of alleles at one locus. In real life, most of the features that we recognize in ourselves and others are controlled by alleles at more than one locus. In fact, most of them have a more complex genetic basis than any that you will encounter in this exercise.

PROCEDURE

You and your partner will work together to determine your own phenotypes and estimate your genotypes for each of 22 traits. These genotypes will be recorded in the Create-a-Baby Table. Then you will each simulate meiosis to select one of your alleles at each locus that is to be passed on to your baby. In each case where a heterozygous locus is involved, you will use a coin toss to determine which allele is to be passed on. When all of these alleles have been recorded, you will be able to predict the phenotype of your imaginary baby. Then you will each draw a baby with this phenotype.

C.3

DETERMINING GENDER

To begin, you and your partner must decide which of you will be Parent 1 (the mom) and which will be Parent 2 (the dad). In humans and many other animals, gender is determined by the **X** and **Y chromosomes**, or **sex chromosomes**. Females have two X chromosomes, so they always pass an X on to each of their babies. Males have an X and a Y, so a father may pass on to his baby either an X chromosome (in which case a girl will be produced) or a Y (in which case a boy will be produced).

Find the Create-a-Baby Table on page 105. Under the Baby's Genotype, record an X to represent the sex chromosome contribution of Parent 1. Now Parent 2 should flip the coin to determine which sex chromosome "he" will contribute (heads = Y and tails = X). Record this under Baby's Genotype and then record the gender of the baby under Baby's Phenotype.

DOMINANT/RECESSIVE TRAITS

On the next page you will find pictures of human phenotypic traits related to facial features. For the purposes of this exercise, we are going to pretend that each of the traits in the top six rows of the diagram is determined by a pair of alleles at a single locus that exhibit a simple dominant/recessive relationship.

1. Where necessary, you and your partner should help each other determine what your own phenotypes are with respect to each of the above traits.

2. Next, each partner needs to determine what genotype corresponds to his/her phenotype for each of these traits. This is easy if you have the homozygous-recessive phenotype: just record the homozygous-recessive genotype in the correct box of the Create-a-Baby Table on page 105. But in each case where you have a dominant phenotype, you will need to flip a coin to determine whether you will record the homozygous-dominant or heterozygous genotype (heads = homozygous-dominant; tails = heterozygous). For example, if you have a mole, you will need to flip the coin to determine whether to record MM or Mm as your genotype at that locus in the second line of the Create-a-Baby Table.

3. Next, each parent needs to determine which allele she or he will pass on to the baby at each locus. In each case where you are homozygous, this is easy: just record the appropriate allele in the correct Baby's Genotype box. But if you are heterozygous, you will need to use the coin again to determine which allele you will pass on (heads = the dominant allele; tails = the recessive allele). Be sure that both parents record the allele they are passing on, so that the baby will be diploid at all loci.

4. Once you have recorded the baby's genotype for all 12 of these features, record the baby's corresponding phenotype.

C.3

CODOMINANT TRAITS

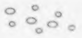
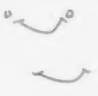
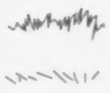
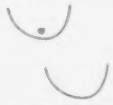
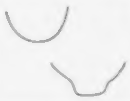
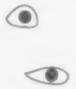
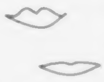
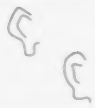
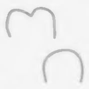
In some cases a pair of alleles exhibit a **codominant** relationship, in which the phenotype of the heterozygote is different from (sometimes intermediate between) that of either homozygote. For the purposes of this exercise, we are going to pretend that the six phenotypic traits that are pictured in the bottom three rows of the diagram are each controlled by a pair of codominant alleles at a single locus.

1. You should proceed in a similar manner with each of these codominant traits as you did with the dominant/recessive traits above. That is, first you should determine your own phenotype, then your genotype, and finally which allele you will pass on to the baby. When in doubt about your own phenotype, choose the one corresponding to the heterozygous genotype.

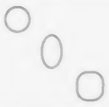
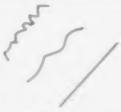
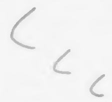
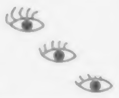
2. After you have determined the baby's genotype for these six codominant traits, record the corresponding phenotype.

C.3

DOMINANT/RECESSIVE TRAITS

Trait	Genotype	Phenotype		Trait	Genotype	Phenotype	
Mole	MM	mole		Cheeks	FF	freckles	
	Mm	mole			Ff	freckles	
	mm	no mole	-none-		ff	no freckles	-none-
Eyebrows (size)	EE	meet		Cheeks	DD	dimples	
	Ee	meet			Dd	dimples	
	ee	do not			dd	no dimples	
Eyebrows (texture)	KK	bushy		Chin	PP	dimple	
	Kk	bushy			Pp	dimple	
	kk	fine			pp	no dimple	
Eyebrows (shape)	BB	arched		Chin (shape)	ZZ	round	
	Bb	arched			Zz	round	
	bb	straight			zz	square	
Eyes (shape)	O	oval		Mouth (shape)	CC	bowed	
	O	oval			Cc	bowed	
	Oo	almond			cc	straight	
Earlobes (shape)	LL	free		Hairline	QQ	widow's peak	
	Ll	free			Qq	widow's peak	
	ll	attached			qq	straight	

CODOMINANT TRAITS

Face (shape)	AA	round		Hair (curliness)	HH	curly	
	Aa	oval			Hh	wavy	
	aa	square			hh	straight	
Nose (size)	NN	large		Eyes (separation)	II	far apart	
	Nn	medium			Ii	medium	
	nn	small			ii	close set	
Lips (size)	GG	full		Eyelashes (length)	JJ	long	
	Gg	medium			Jj	medium	
	gg	thin			jj	short	

C.3

MULTIGENIC TRAITS

In actuality, most human heritable traits are determined not by a pair of alleles at a single locus but by a combination of alleles at two or more loci. Such traits are said to be **multigenic**. For example, it is believed that human skin color is determined by the combination of alleles present at seven or more different loci. Thus, as we can see by looking around the world, enormous variation in skin color is present in the human population, even within what is usually considered to be a single ethnic group. Inheritance patterns for hair and eye color are only slightly less complex.

In this exercise, however, we will simplify the situation by pretending that each of these three color phenotypes is determined by only a pair of alleles at two loci, as follows:

Hair color			Eye color			Skin color		
Genotype		**Phenotype**	**Genotype**		**Phenotype**	**Genotype**		**Phenotype**
RR	*SS*	Black	*TT*	*UU*	Deep brown	*VV*	*WW*	Black
RR	*Ss*	Black	*TT*	*Uu*	Deep brown	*VV*	*Ww*	Dark brown
RR	*ss*	Red	*TT*	*uu*	Brown	*VV*	*ww*	Light brown
Rr	*SS*	Brown	*Tt*	*UU*	Greenish brown	*Vv*	*WW*	Medium brown
Rr	*Ss*	Brown	*Tt*	*Uu*	Light brown	*Vv*	*Ww*	Beige
Rr	*ss*	Blond	*Tt*	*uu*	Gray blue	*Vv*	*ww*	Light beige
rr	*SS*	Dark blond	*tt*	*UU*	Green	*vv*	*WW*	Olive
rr	*Ss*	Blond	*tt*	*Uu*	Dark blue	*vv*	*Ww*	Fair
rr	*ss*	Light blond	*tt*	*uu*	Pale blue	*vv*	*ww*	Ivory

1. You should proceed with these traits in the same general way that you did with the dominant/recessive traits. If you are in doubt about which of the two combinations of alleles you should use to account for your own phenotype, flip a coin.

2. After you have determined your own genotype for each of these traits, determine which allele at each locus you will pass on to the baby. In every case where you are heterozygous, flip a coin to determine which allele you will pass on (heads = capital letter allele; tails = small letter allele).

Remember, like you, the baby should have four alleles for each of these traits, two different letters from each parent.

DRAWING ON YOUR GENETIC RESOURCES

When you and your partner have finished filling in the Create-A-Baby Table on the next page, name the baby. Then each of you should draw its face, incorporating as many aspects of its recorded phenotype as possible. Remember that it's supposed to be a baby, not an adult, that you are drawing. Do not look at your partner's drawing until both of you are finished. Then compare your two drawings and see how similar they look. If they look quite different, you can consider it evidence for a concept you will encounter shortly: namely, that the same genes can produce different phenotypes in different environments.

C.3

Name ______________________________

Date ________________ Hour __________

CREATE-A-BABY TABLE

Parent 1's name (the Mom)__________ ________________

Parent 2's name (the Dad)__________ ________________

Baby's name ________________ ________________

Trait	Parent 1's Genotype	Parent 2's Genotype	Baby's Genotype	Baby's Phenotype
Gender				
Mole				
Eyebrows (size)				
Eyebrows (texture)				
Eyebrows (shape)				
Eyes (shape)				
Earlobes (shape)				
Cheeks (freckles)				
Cheeks (dimples)				
Chin (dimple)				
Chin (shape)				
Mouth (shape)				
Hairline				
Face (shape)				
Nose (size)				
Lips (size)				
Hair (curliness)				
Eye (separation)				
Eyelashes (length)				
Hair (color)				
Eyes (color)				
Skin (color)				

C.3

Name ______________________

Date ______________ Hour ________

CREATE-A-BABY REVIEW

1. Define each of the following terms.

   chromosome: ______________________

   codominant: ______________________

   diploid: ______________________

   haploid: ______________________

   meiosis: ______________________

   multigenic: ______________________

   recombination: ______________________

2. What was the probability that you and your partner would produce a boy? A girl? Explain.

   ____

3. Explain how it is possible for your baby to have a visible trait that neither you nor your partner have.

   ____

4. If you and your partner repeated this exercise and produced another imaginary baby, do you think it would look just the same as the one you produced already? Explain.

   ____

5. A woman who is heterozygous for the chin-dimple trait marries a man without a chin dimple. What are the possible genotypes and phenotypes of their children?

   ____

6. What is the probability that the man and woman discussed in the preceding question will have a baby with a chin dimple?

   ____

C.3

Name

Date Hour

7. A man and a woman who are both heterozygous for two traits, the cheek-dimple and the chin-dimple traits, get married. What is the probability that they will have a baby that has cheek dimples but not a chin dimple? (If you have trouble answering this question, check out section C.4.)

8. What is the probability that a man with dark blonde hair and a woman with red hair will have a baby with brown hair?

C.4

Using a More Complicated Punnett Square

INTRODUCTION

In Exercise C.2 we learned how to use a Punnett square to figure out the probability that a baby would have a trait that was determined by alleles at a single locus. A Punnett square can also be used to determine the probability that a child will have a trait or combination of traits that involves alleles at two or more loci. It just gets a bit more complicated.

PROCEDURE

Let's see how to use a Punnett square to answer a question such as the following: When a man with an oval face and wavy hair marries a woman with an oval face and wavy hair and they have a baby, what is the probability that their baby will have a square face and curly hair? We follow a procedure that is similar to the one we followed before, except that our diagram must be larger to make room for alleles at two loci.

1. From the diagrams that illustrated the codominant traits, we find that the genotypes of the parents are as follows:
   Man with oval face and wavy hair: ***Aa Hh***
   Woman with oval face and wavy hair: ***Aa Hh***
2. Now we need to draw a diagram that has enough boxes along each edge to accommodate all of the different kinds of gametes each parent can produce. The rule for this is that each edge of the diagram should have 2^n boxes, where n is the number of loci being considered. In our first Punnett square, with only one locus being considered, we only needed 2^1 or two boxes on a side. But here, with two loci to be considered, we need 2^2 or 4 boxes along each edge of the large square.

		Eggs produced by an *AaHh* female			
		AH	*Ah*	*aH*	*ah*
Sperm produced by an *AaHh* male	*AH*				
	Ah				
	aH				
	ah				

Figure 1

3. Above the squares along the top row we write the genotypes of all the different kinds of eggs the woman can produce with respect to alleles at the *a* and *h* loci (*AH*, *Ah*, *aH*, and *ah*) as done in fig. 1.

4. Beside the squares along the left side, we write the genotypes of the kinds of sperm the man will produce (fig. 1). (In this case, it happens to be the same combinations of alleles as for the eggs, because he happens to have the same genotype as his wife in this particular example. But this is not always the case, of course.)

5. In each of the smaller boxes, we now write the genotype we will get when we combine an egg of the type shown above with a sperm of the type shown to the left (fig. 2).

6. Below these genotypes, we write the corresponding phenotypes (fig. 2).

7. Now we determine how many of the boxes contain the phenotype that the question asked about, namely, a square face and curly hair. We see that only one of the 16 boxes (shaded in the diagram) is labeled "square curly." Thus we can conclude that the prospect of these two parents having a baby with a square face and curly hair is 1-in-16. From this same diagram we can also determine the probability that each of the other possible phenotypes would appear in the progeny. For example, the probability of a baby resembling both of the parents with respect to these two traits (oval face and wavy hair) is 4-in-16 (4/16) or 1-in-4 (1/4).

Eggs produced by an *Aa Hh* female

Sperm produced by an *Aa Hh* male

	AH	***Ah***	***aH***	***ah***
AH	*AA HH* Round Curly	*AA Hh* Round Wavy	*Aa HH* Oval Curly	*Aa Hh* Oval Wavy
Ah	*AA Hh* Round Wavy	*AA hh* Round Straight	*Aa Hh* Oval Wavy	*Aa hh* Oval Straight
aH	*Aa HH* Oval Curly	*Aa Hh* Oval Wavy	aa HH Square Curly	*aa Hh* Square Wavy
ah	*Aa Hh* Oval Wavy	*Aa hh* Oval Straight	*aa Hh* Square Wavy	*aa hh* Square Straight

Figure 2

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CHAPTER 2

Passing Traits from One Generation to the Next

SECTION D

How Are Genetic Experiments Actually Performed?

D.1

A Colorful Experiment in Yeast Genetics

INTRODUCTION

OK, FOLKS, ENOUGH IMAGINARY organisms for a while. Let's do a real genetic experiment, with a real, live model organism. The model organism we will use is baker's yeast. Yes, this is the same organism that the bakers of Bunny Bread use to make that spongy white stuff for your peanut butter and jelly sandwiches! Baker's yeast is a unicellular fungus with a life cycle that at first seems very different from that of the more familiar animals and plants (see page 113).

As different as it looks at first glance however, you will notice that the yeast life cycle does resemble the life cycle of animals and plants in several very important regards. It involves haploid and diploid cells and thus meiosis and recombination. Indeed, yeast follows the same basic rules of inheritance that we do, even though its haploid cells bear no resemblance to the sperm and egg cells of animals.

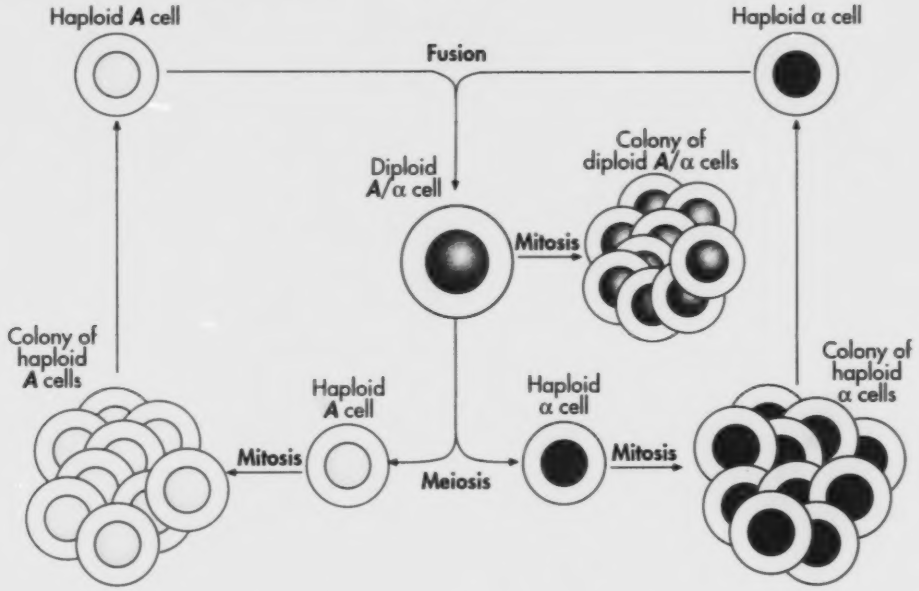
Because yeast can complete its life cycle in less than a day (under optimum conditions), it can be used to perform many different genetic experiments in a very short time. As a result, it is one of the most popular model organisms for geneticists who are interested in studying the basic principles of genetics that apply to all eukaryotic organisms.

In this exercise, we will use red and white strains of yeast to study a simple genotype-phenotype relationship that until now we had only encountered as a theoretical concept. In addition to strains that differ in color, we will also need strains that differ in mating type so that they will be able to fuse to make diploids. Yeast geneticists call the two mating types of yeast ***a*** (small a) and ***α*** (alpha). But to be sure that we do not confuse the two strains in our experiment, we will use the terms *A* (capital A) and ***α*** (alpha). In a sense, the two mating types, ***A*** and ***α***, are to yeast as males and females are to animals.

Your job will be to formulate a hypothesis about the genetic basis for the color difference that you will observe between the haploid strains you will cross, use that hypothesis to make a prediction about what color the diploids will be, and then mate four strains of yeast to test your hypothesis.

D.1

THE LIFE CYCLE OF YEAST


Haploid *A* cell
Haploid α cell
Fusion
Diploid *A*/α cell
Colony of diploid *A*/α cells
Mitosis
Colony of haploid *A* cells
Haploid *A* cell
Haploid α cell
Colony of haploid α cells
Mitosis
Meiosis
Mitosis

Haploid and diploid yeast cells look similar, and both can divide mitotically to form large colonies. Haploid cells come in two mating types, which we will call *A* and α. As long as these two mating types are kept apart, they continue to grow and divide in the haploid state. But if they make contact, they fuse to form an *A*/α diploid. Under most conditions, the *A*/α diploids will grow and divide continuously, forming colonies.

Under certain nutritional conditions, however, *A*/α diploids undergo meiosis to produce new *A* and α haploids. If the *A* and α cells are not separated at once, they fuse again to make new *A*/α diploids. At first this might seem like a waste of effort, but it is not. Because various alleles will have been sorted out and recombined at random in the process, the new generation of *A*/α diploids will include individuals that are genetically different from those in the earlier generation, and one or more of these new variants might be better adapted to the nutritional conditions that now exist.

MATERIALS

For each group of four student(s):

1 petri dish containing yeast growth medium
marking pen
a packet of sterile toothpicks
culture of Red Mating type **A** yeast
waste container
culture of Red Mating type α yeast
culture of White Mating type **A** yeast
culture of White Mating type α yeast
disinfectant

D.1

PROCEDURE

Day 1: Growing Your Haploid Yeast Cells

Do not open your petri dish until instructed to do so.

1. Leave your petri dish upside down. Draw four lines with the marking pen, as shown in the diagram. Write your name and class hour at the bottom. Write "Mating Type *A*" across the top and "Mating Type *α*" along the left side. Then write the letters indicating the colors of the yeast strains (R for red and W for white) in the spaces indicated (fig. 1).

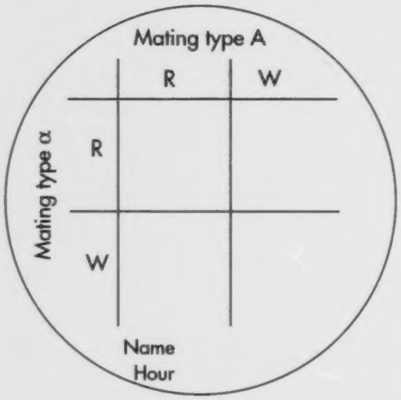


Figure 1

2. Shake your box of toothpicks gently so that a toothpick comes part way out of the corner hole. Remove the toothpick carefully, without touching the other end. If any toothpicks fall out of the box, ignore them and throw them away later.

3. Select the culture dish that has the Red Mating type *A* yeast strain on it. Leave it upside down. While holding your toothpick in one hand, use your other hand to lift the bottom half of the dish that contains the Red Mating type *A* culture. Do not turn it over. Now gently scrape the end of your toothpick across the culture to pick up a small quantity of yeast on the toothpick. Put the stock culture back on its lid.

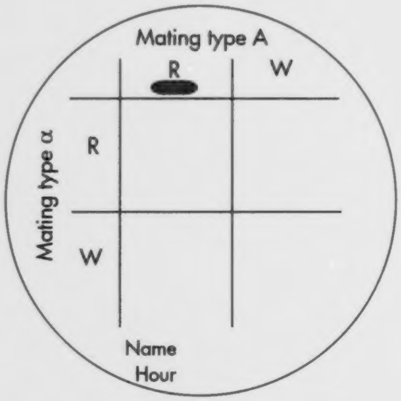


Figure 2

4. Lift the bottom of your experimental dish (the one you marked in step 1). Do not turn it over. Carefully transfer the yeast cells to your dish by rubbing your toothpick on the agar in the box that contains R under the Mating Type *A* (fig. 2). Put the plate back on its lid. Discard the toothpick.

5. Repeat steps 2-4 with each of the other three strains of yeast. Use a fresh toothpick for each strain, and place each kind of the yeast in the space indicated on fig. 2.

6. Put your stock cultures and your experimental culture dish in the places indicated by the teacher.

7. Wipe your work area with disinfectant and wash your hands.

Day 2: Making the Genetic Crosses

Do not open your petri dish until instructed to do so.

1. Examine your petri dish carefully. Have all four of your yeast colonies grown well? Record your observations on the Day 2 Work Sheet.
2. With the dish still closed, draw four circles and number them 1 to 4 (fig. 3).
3. Get a sterile toothpick. Lift the agar-containing part of the petri dish, and — while keeping the dish upside down — gently rub the Red Mating type *A* colony with the end of the tooth pick. Now rub the toothpick on the agar lightly in the center of circle 1. You want to deposit only a small number of cells on the agar — barely enough to see. If you have more than this, try to scrape off the excess. Discard the toothpick in the waste jar.

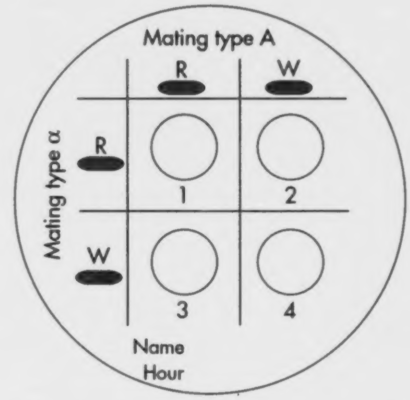


Figure 3

4. With a new sterile toothpick gently rub the Red Mating type α colony and then rub the toothpick in the same region of circle 1 where you rubbed with the Red Mating type *A* toothpick. Mix the two kinds of yeast cells together with the toothpick, but avoid stabbing the agar. Discard the toothpick in the waste jar.
5. Repeat steps 3 and 4 for the three empty circles, so as to cross the White Mating type *A* and Red Mating type α strains in circle 2, the Red Mating type *A* and White Mating type α strains in circle 3, and the two white strains in circle 4.
6. Place your petri dish in the incubator or where your teacher directs you.
7. Wipe your work area with disinfectant. Wash your hands.
8. Complete the Day 2 Work Sheet.

D.1

Name ______________________________

Date ____________________ Hour __________

DAY 2 WORK SHEET

1. Describe the appearances of the four colonies of haploid yeast cells at the beginning of class on Day 2.

   ____

   ____

2. Formulate a hypothesis about the genetic difference that causes the difference in appearance of the red and white yeast strains.

   ____

   ____

3. Which color trait do you think will be dominant, or do you think that they will be codominant? Why?

   ____

   ____

4. Based on the above hypotheses, what do you predict the color phenotypes of the diploids will be in the following four crosses that you have set up?

   Red Mating type *A* x Red Mating type *α* ______________________

   Red Mating type *A* x White Mating type *α* ______________________

   White Mating type *A* x Red Mating type *α* ______________________

   White Mating type *A* x White Mating type *α* ______________________

D.1

Name ______________________________

Date ____________________ Hour ____________

Day 3: Observing Your F1 (A/α Diploids)

1. Get your petri dish and observe your results.

   Even though there are no sperm and eggs involved, a Punnett square diagram can be used to record and analyze the results of yeast crosses such as the ones you performed. Use your observed results to fill in the blanks on the Punnett square on the Day 3 Work sheet on the next page.

2. Are any of your results unclear? If so, indicate which ones, describe what you see, and provide a good explanation for these results.

   ____

   ____

3. If any of your test circles contain a mixture of red and white cells, incubate the dish for another day and see if things change. If they do, be sure to record this on your Day 3 Work Sheet.

4. When you are through working with your cultures, spray disinfectant in your dish, tape it shut, and dispose of it as instructed by your teacher. Wipe your work area with disinfectant. Wash your hands.

5. Finish filling out the Day 3 Work Sheet.

D.1

Name ______________________________

Date ____________________ Hour __________

DAY 3 WORK SHEET

1. Use the results from your yeast crosses to fill in the blanks on the diagram below:

Mating type **A**

Mating type α	**R**	**W**
R	Genotype ______ Phenotype ______	Genotype ______ Phenotype ______
W	Genotype ______ Phenotype ______	Genotype ______ Phenotype ______

2. What ratio of phenotypes did you observe as a result of the four crosses you performed?

____

3. What does this indicate about which allele is dominant and which is recessive?

____

4. Is this what you predicted on your Day 2 Work Sheet?

____

5. In the table below, list what you predicted and what you observed for each of the four crosses.

Cross	**Predicted Phenotype of Diploid**	**Observed Phenotype of Diploid**
Red Mating type ***A*** by Red Mating type ***α***		
Red Mating type ***A*** by White Mating type ***α***		
White Mating type ***A*** by Red Mating type ***α***		
White Mating type ***A*** by White Mating type ***α***		

D.1

Name ______________________________

Date ____________________ Hour ____________

6. If your predicted and observed phenotypes do not agree, how can you account for that, and can you propose a good hypothesis to account for the results you actually observed?

7. If you have come up with a new hypothesis, can you think of a way to test it?

D.2

Experimenting with Wisconsin Fast Plants: Phase 1

INTRODUCTION

MOST PLANTS ARE NOT suitable for use in classroom biology projects, because most plants grow slowly, take months or years to produce seeds that are ready to be planted, and get so large that there is not room for more than a few of them in a classroom.

Paul Williams, a plant biologist at the University of Wisconsin, set out to change all that and develop plants that would be convenient and fun for students to grow and study in the classroom. To achieve his goal, he selected the most rapidly flowering plants he could find, crossed them with one another, and continued this selective breeding for several plant generations. The resulting plants are the Wisconsin Fast Plants, some of which you will use in this exercise. These plants flower in two weeks, produce mature seed in five to six weeks, and are so small that it is possible to grow hundreds of them in a classroom.

Another name for Wisconsin Fast Plants is **rapid-cycling brassicas**. *Brassica* is the genus of plants that includes cabbage, broccoli, cauliflower, turnips, mustard greens, collards, and many other popular food plants. The Fast Plants are members of the species *B. rapa*, the *Brassica* species from which bok choi and Chinese cabbage were derived.

Initially you will use the Fast Plants just to study the plant life cycle. You will plant the seeds, watch the plants germinate and grow, and learn how to transfer pollen from one plant to another when the flowers appear. Then you will watch the seeds develop from the flowers you have pollinated and eventually collect the seeds. By the time your seeds are mature, however, you will find out that they are part of a more extensive and more interesting experiment. You will be given more information about that when the time comes. But for now, let s just get our seeds planted and watch our garden grow.

D.2

MATERIALS

For the class (Day 1):
- 1 container of potting mix
- 2 spoons
- 1 ruler
- 1 bottle of 1/8 X Peter's Professional Fertilizer
- masking tape
- felt-tip marking pens

For each group of four students (Day 1):
- 1 film-can growth system (Fig. 4)
- 1 water bottle
- Fast Plant seeds in a small envelope

For each set of four student groups (Day 1):
- 1 plant lighthouse
- 1 piece of foil-covered cardboard
- 6 pieces of 1 inch thick insulating foam

For each group of four students later on:
- 14 25-cm bamboo skewers to be used as plant stakes
- 14 split-ring ties to hold plants to stakes
- 4 dried bees
- 4 toothpicks
- Duco fast-drying cement
- 1 brown paper lunch bag
- 1 small envelope

PROCEDURE

You will grow your plants in a film-can growth system that consists of several parts (Fig. 1-4). The film cans will hold your plants and the soil in which they grow; the other pieces form an automatic watering system that will keep the soil moist and give your plants a constant supply of water and nutrients.

Put a piece of masking tape on the bottom chamber of your growth system (the nutrient reservoir) and with the marking pen write your group name and your class period on the tape. (Make sure that the tape does not overlap the clear stripe on the bottom chamber, because you will need to look through this stripe to monitor the fluid level.)

1. **Preparing the growth system and planting the seeds (Day 1)**

   a. Make sure that a wick is about half in and half out of the bottom of each can (Fig. 1).

   b. Hold your film-can cluster over the container of soil and fill each can loosely. Tap the film cans on the side to help the soil settle, but DO NOT PRESS THE SOIL INTO THE CANS.

   c. Use the ruler to scrape off any excess soil so that each can is filled up to but not over the top (Fig. 2).

   d. Make sure that both ends of the long wick hang out of your slotted tray so that they will hang freely into the nutrient reservoir (Figs. 3-4).

   e. Hold the film-can cluster over the slotted tray. Turn your water bottle upside down and squeeze gently to direct a stream of water at each film can. Water each can until water drips from its wick (Fig. 2). (This will cause the soil to settle somewhat.)

   f. Water the wick in the slotted tray until it is saturated and water runs freely into the nutrient reservoir.

   g. Place four seeds, evenly spaced, on the surface of the soil in each film can.

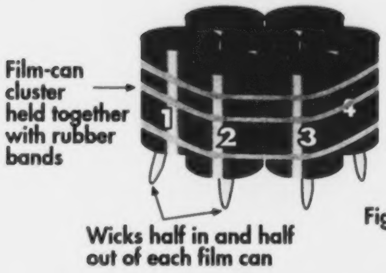

Film-can cluster held together with rubber bands
1
2
3
4
Wicks half in and half out of each film can

Figure 1

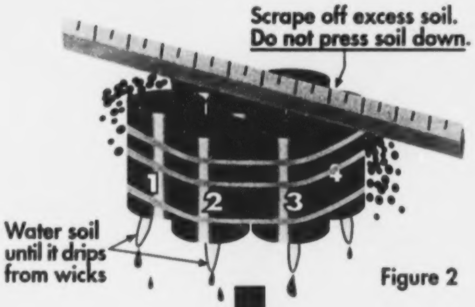

Scrape off excess soil. Do not press soil down.
1
2
3
4
Water soil until it drips from wicks

Figure 2

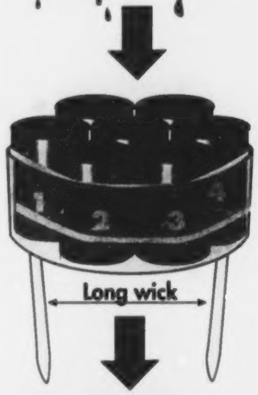

1
2
3
4
Long wick

Figure 3

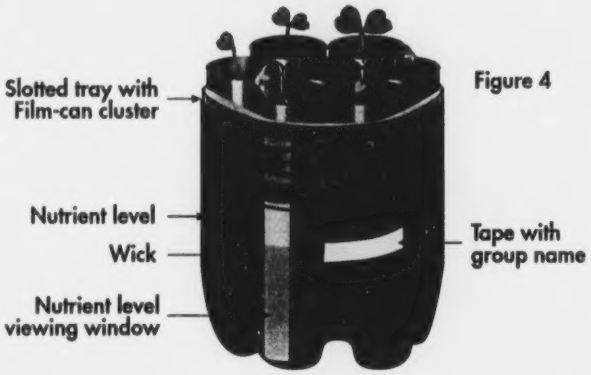

Slotted tray with Film-can cluster
Nutrient level
Wick
Nutrient level viewing window
Tape with group name

Figure 4

h. Take your film-can cluster back to the container of potting mix. Carefully sprinkle soil over the seeds to fill each can to the top. Level the soil with the ruler. DO NOT PRESS THE SOIL INTO THE CANS.

i. Add 1/8 X Peter s Professional Fertilizer (as a nutrient solution) to the nutrient reservoir until the level is just below the bottom of the slotted tray.

j. Place your film-can cluster in the slotted tray. Water each can gently until water begins to run out of it and through the slots into the nutrient reservoir.

k. Your plants will be grown in a plant lighthouse (Fig. 5). Take your growth system to the plant lighthouse to which your teacher assigned you.

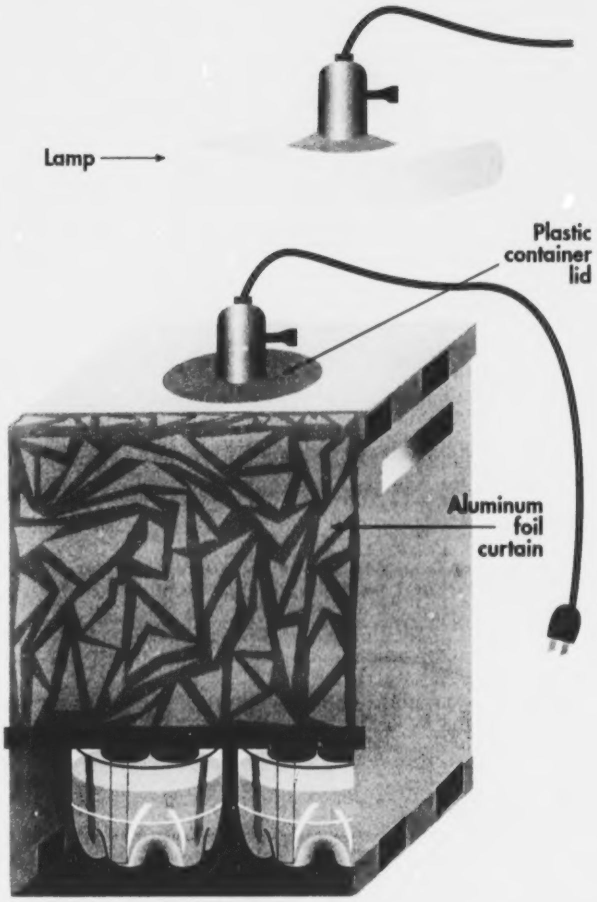


Figure 5

l. If the following has not already been done, stack six foam blocks on the floor of the lighthouse and then place the foil-covered cardboard on top of the stack.

m. Place your growth system on top of the foil-covered cardboard, toward one corner of the lighthouse.

2. After Day 1

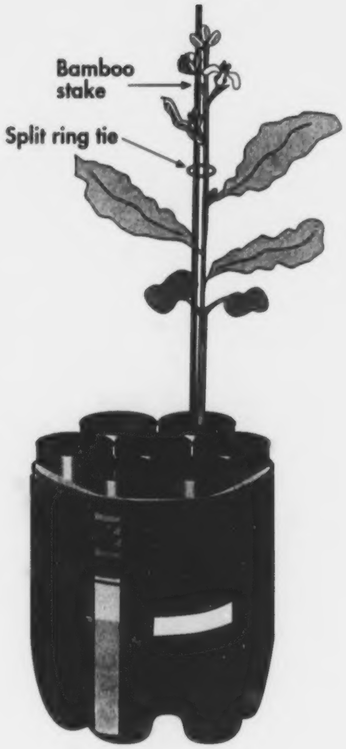


Figure 6

a. **Days 2-4** Use your water bottle to sprinkle the surface of the soil in each film can. Record how many plants emerge in each can and when they emerge.

b. **Every day after Day 4** Check the liquid level in your nutrient reservoir. Add 1/8 X Peter's nutrient as necessary to keep liquid level up near (but never above) the bottom of the upper chamber. A good way to do this is to move the film-can cluster to one side, slowly pour the Peter's nutrient into the upper chamber and let it run thorough to the bottom. This will keep all the wicks wet. It is particularly important to fill the reservoir just before a weekend or holiday.

   Observe your plants regularly and keep a record of their progress. Record when they look like each of the stages pictured on your work sheets (see pages S121-S123). After most of your plants reach one of the pictured stages, fill in the date and the number of days that have passed since you planted the seeds. It would also be a good idea to keep a journal in which you describe what you see each time you observe the plants.

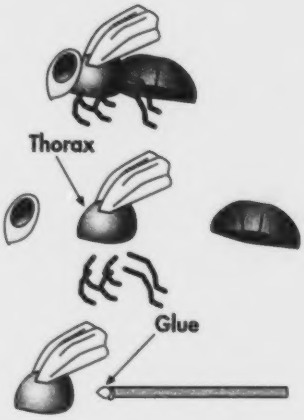
c. **Day 4 or 5** If there are fewer than two seedlings in any of your film cans, you may transplant seedlings from cans that have more than two. To do this, use a pencil to make a depression in the spot to which you wish to move a seedling. Carefully lift a seedling using your fingers or a pair of forceps, and tuck its roots into the depression you have just made. Use the pencil to tuck soil around its roots, and sprinkle the soil lightly with water.

d. **Day 7 or 8** Remove all but the two healthiest-looking seedlings from any film can that still has more than two. Use a pair of scissors to snip off each unwanted plant just above ground level.

e. **Days 8-14** As your plants grow taller, use bamboo stakes to support them and prevent them from falling over. Insert a stake near a plant; then attach the plant to the stake with a split ring tie (Fig. 6).

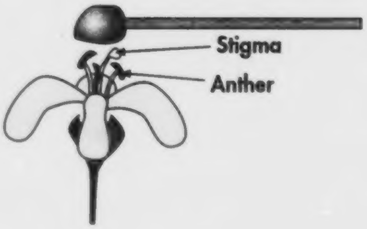
As the plants grow, remove foam sheets from the lighthouse as necessary to prevent the tips of the plants from getting closer than 10 cm to the light.

3. **Making "bee sticks" and using them to pollinate plants**

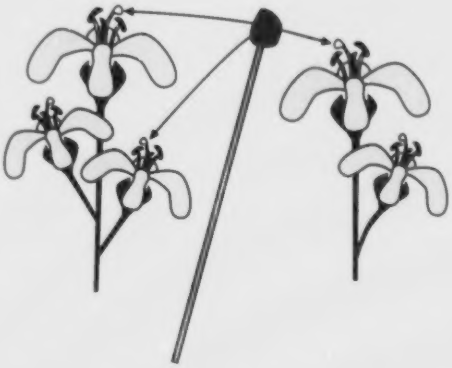


a. **Day 11 or 12** Using a dry, dead bee and a toothpick, make a bee stick as follows:

Hold the bee by the wings while you remove the abdomen, the head, and the legs. (Don t worry. This bee has long since lost the ability to sting you.) What you have left is the thorax with the wings attached.



Place a small drop of fast-drying glue on the tip of the toothpick and stick it in the hole left by the removal of the abdomen or the head. Stick the other end of the toothpick into a foam sheet and leave your bee stick overnight to dry. The next day, after the glue is dry, remove the wings from the thorax.



b. **Days 13 to 18** After flowers on your plants open, cross-pollinate the plants as follows:

Use the bee stick to pick up pollen from the anthers of one flower. (The anthers are the male parts of the flower that are arranged around the central stigma, which is the female part of the flower.) To pick up more pollen, rotate the beestick as you touch an anther.

Now transfer the pollen to the stigmas of flowers on several different plants; this is called cross-pollination. Brassica flowers are self-incompatible, which means that pollen from one plant is unable to fertilize flowers on that same plant but usually can fertilize flowers on another plant.

The more plants you involve in such cross-pollination, the more likely you are to obtain a lot of healthy seeds. (So all students in the group should take turns pollinating.)

Repeat the cross-pollination process on at least three successive days. Try to use a different cross-pollination pattern each day.

You will harvest your seeds 20 days after your last cross-pollination. Add a tape label to your nutrient reservoir that indicates what the date will be.

4. **Harvesting seeds**

Seedpods and seeds begin to develop and grow shortly after the flowers have been successfully pollinated, but it takes 20 days for the seeds to become mature.

a. 20 days after the last day you pollinated, remove your film-can cluster from the watering system. Put the cluster first on a paper towel and then back in the lighthouse so that the plants can dry out.

Rinse out both chambers of the watering system and let them dry out, so they will be ready for reuse.

b. After the plants have dried for about five days, the seed pods should be crisp and brown. As soon as they are, cut the seed pods off the plants one at a time and put them in the paper bag. Seal the bag with staples or tape and then crush the pods.

c. Unseal the bag and carefully dump its contents into a shallow container or onto a sheet of white paper. Pick off and discard large pieces of debris. Blow gently to blow off small pieces of debris.

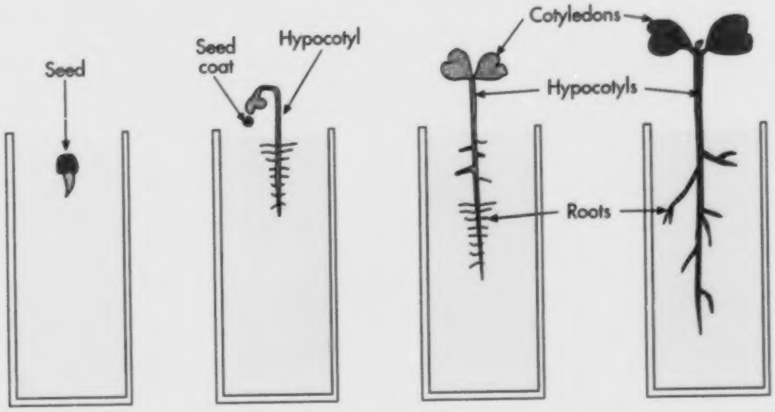
d. Transfer as many seeds as you can to a small envelope. Label the envelope with your names and your class hour. Store the seeds in a safe, dry place until your teacher announces that it is time to start Phase 2 of the Fast Plants experiment.

e. Clean out your film-can cluster and prepare it for reuse. If you have lost any wicks, ask your teacher for a piece of capillary material to make new wicks.

D.2

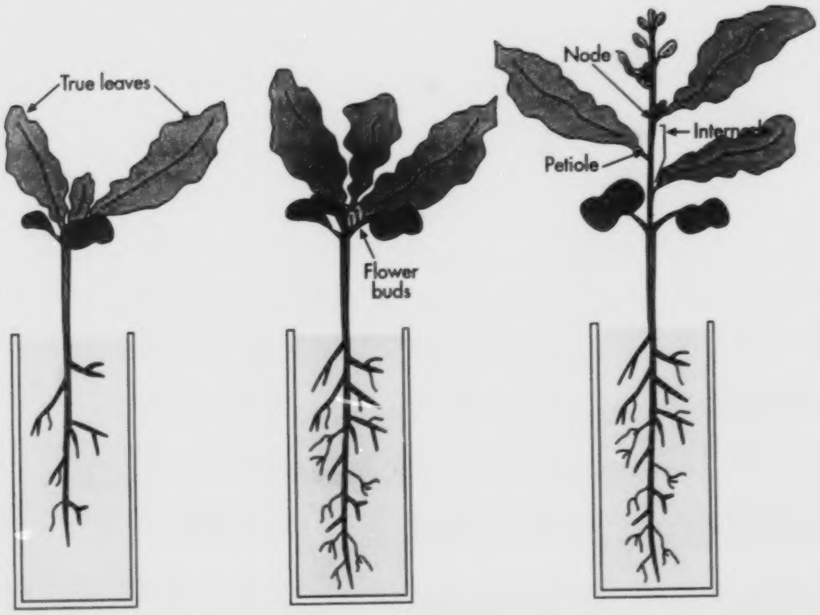
Name ______________________________

Date ____________________ Hour ____________


Seed
Seed coat
Hypocotyl
Cotyledons
Hypocotyls
Roots

Calendar date ________ ________ ________ ________

Days since planting ________ ________ ________ ________


True leaves
Flower buds
Node
Interno
Petiole

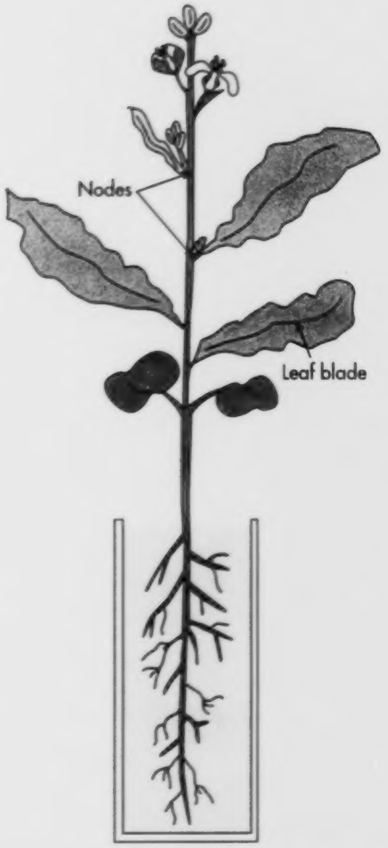
Calendar date ________ ________ ________

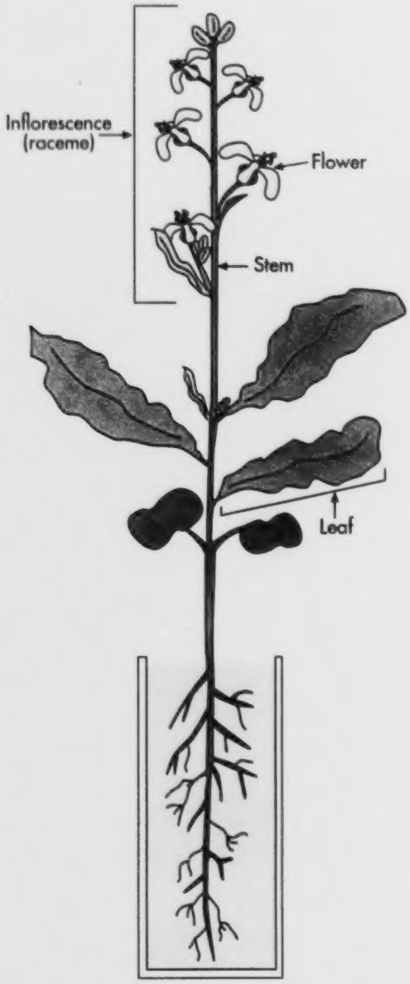
Days since planting ________ ________ ________

D.2

Name ______________________________

Date ________________ Hour __________


Nodes
Leaf blade

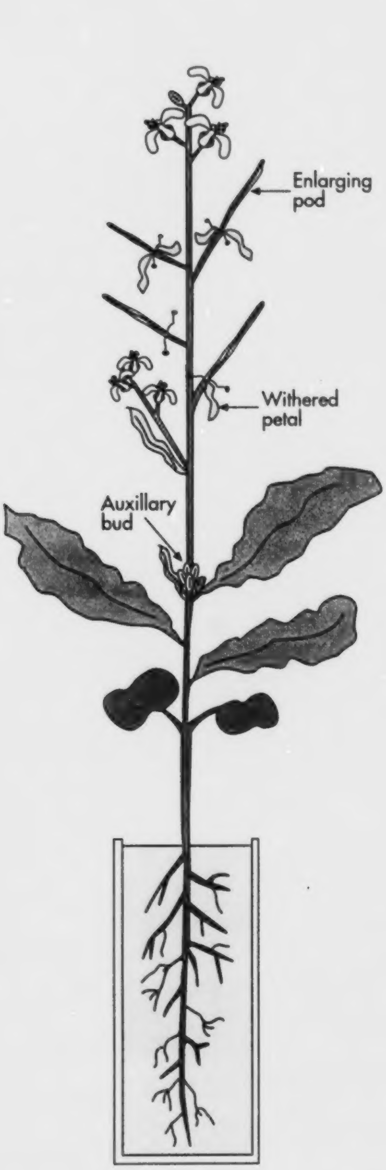

Inflorescence (raceme)
Flower
Stem
Leaf

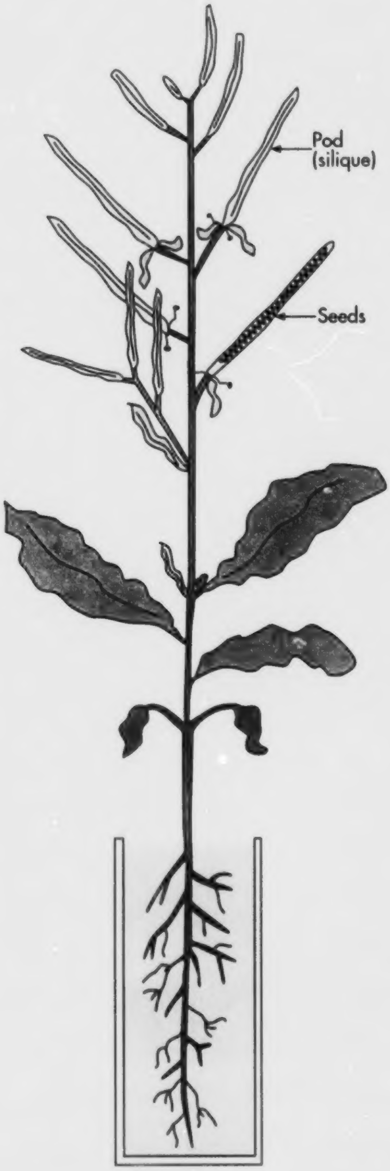
Calendar date	__________	__________
Days since planting	__________	__________

D.2

Name ____________________

Date ______________ Hour __________


Enlarging pod
Withered petal
Auxillary bud


Pod (silique)
Seeds

Calendar date __________ __________

Days since planting __________ __________

D.2

Experimenting with Wisconsin Fast Plants: Phase 2

INTRODUCTION

NOW IT CAN BE TOLD! The seeds that you planted at the beginning of Phase 1 were not produced by just any Fast Plants. You were given seeds that had been produced by cross-pollinating two plants that differed from one another with respect to two conspicuous traits.

Organisms that are produced by a cross between genetically distinguishable parents are called **hybrids**. When those parents differ in two heritable traits, the cross between them is called a **dihybrid cross**. The seeds you received were the result of such a dihybrid cross.

The principal purpose of Phase 2 of the Fast Plants exercise is twofold. First you are to determine what distinguishing traits were present in the plants that generated the seeds you initially planted, and then you are to determine what sort of inheritance patterns those traits exhibit.

Biologists have a set of terms that they use to keep track of the generations in such genetic experiments. The plants with which the experiment begins are members of the **parental**, or **P**, generation. Their offspring are called the **first filial**, or **F_1**, generation. [The Latin words for *son* and *daughter* are *filius* and *filia*, respectively.] The progeny of the F_1 generation are called the **second filial**, or **F_2**, generation.

The seeds with which you began Phase 1 were produced by crossing two visibly different P-generation plants. We will call these two different P-generation plants PA and PB. The plants that you grew initially constituted the F_1 generation. You cross-pollinated your F_1 plants to produce the seeds that you will soon use to produce plants of the F_2 generation. The objective of this experiment is to determine which of the visible traits present in the P-generation plants (but not visible in your F_1 plants) will reappear in the F_2 plants. Because you may use a mathematical method to test certain genetic hypotheses, it is important to germinate a substantial number of F_2 seeds and to keep careful track of how many plants of each different phenotype you see.

D.2

MATERIALS

For the class:
- 1 container of potting mix
- 2 spoons
- 1 ruler
- 1 bottle of 1/8 X Peter s Professional Fertilizer

For each group of four students:
- 1 film-can growth system
- 1 water bottle
- 1 envelope with 6 PA seeds
- 1 envelope with 6 PB seeds
- 1 envelope with 6 double-mutant seeds
- the F_2 seeds collected in Phase 1

For each set of four groups of four students:
- 1 plant lighthouse
- 1 piece of foil-covered cardboard
- 6 pieces of 1 inch thick styrofoam

D.2

PROCEDURE

You will follow the same basic procedures as for Phase 1 for the first few days, except that you will plant different kinds of seeds in different film cans.

1. **Preparing the growth system and planting the seeds (Day 1)**

   a. Make sure that a wick is about half in and half out of the bottom of each can (Fig. 1).

   b. Hold your film-can cluster over the container of soil and fill each can loosely. Tap the film cans on the side to help the soil settle, but DO NOT PRESS THE SOIL INTO THE CANS.

   c. Use the ruler to scrape off any excess soil so that each can is filled up to, but not over the top (Fig. 2).

   d. Make sure that both ends of the long wick hang out of your slotted tray and hang freely into the nutrient reservoir.

   e. Hold the film-can cluster over the slotted tray. Turn your water bottle upside down and squeeze gently to direct a stream of water at each film can. Water each can until water drips from its wick (Fig. 2). (This will cause the soil to settle somewhat.)

   f. Water the wick in the slotted tray until it is saturated and water runs freely into the nutrient reservoir.

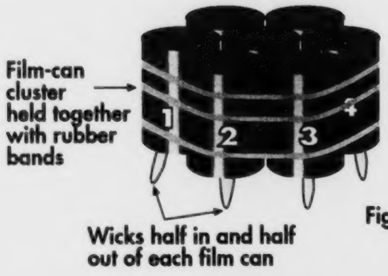

Film-can cluster held together with rubber bands
1
2
3
4
Wicks half in and half out of each film can

Figure 1

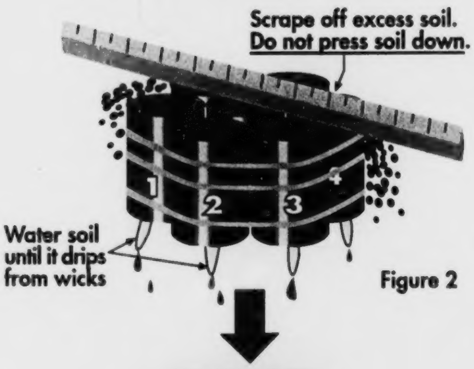

Scrape off excess soil. Do not press soil down.
1
2
3
4
Water soil until it drips from wicks

Figure 2

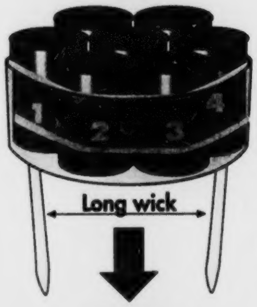

1
2
3
4
Long wick

Figure 3

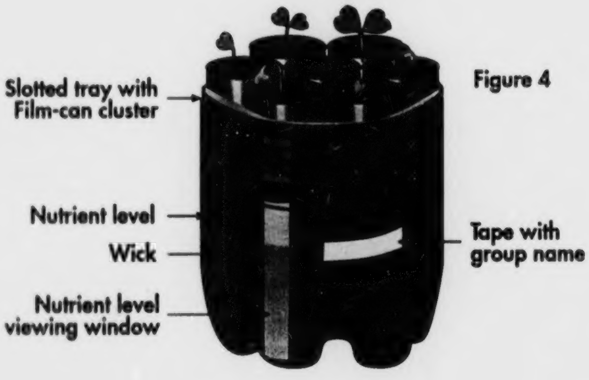

Slotted tray with Film-can cluster
Nutrient level
Wick
Nutrient level viewing window
Tape with group name

Figure 4

g. Place the film-can cluster in the slotted tray. Paying attention to the numbers on your film cans, place six seeds, evenly spaced, on the surface of the soil in each film can, according to the following plan:

Can 1: six PA seeds.
Can 2: six PB seeds.
Can 3: six double-mutant seeds.
Cans 4-7: six of your F_2 seeds in each can. If you do not have enough F_2 seeds, try to get some from another group. If you have extra F_1 seeds, share with those who need more.

h. Take your film-can cluster back to the container of potting mix. Carefully sprinkle soil over the seeds to fill each can to the top. Level the soil with the ruler. DO NOT PRESS THE SOIL INTO THE CANS.

i. Add 1/8 X Peter s nutrient solution to the nutrient reservoir until the level is just below the bottom of the slotted tray.

j. Place your film-can cluster in the slotted tray. Water each can gently until water begins to run out of it and through the slots into the nutrient reservoir.

k. Take your growth system to your plant lighthouse. If the following has not already been done, stack six foam blocks on the floor of the lighthouse and then place the foil-covered cardboard on top of the stack.

l. Place your growth system on top of the foil-covered cardboard, toward one corner of the lighthouse.

2. **After Day 1**

a. **Days 2-4** Use your water bottle to sprinkle the surface of the soil in each film can.

b. **Days 3-5** Observe your film cans daily and record what you see on the Observations pages. Record how many seedlings emerge in each can and when they emerge. Each day after that observe all seedlings carefully, looking for significant differences among them. Compare your plants to the photographs of mutant Fast Plants or to the live Fast Plant mutants that your teacher put on display. By Day 5, assign phenotypes to all the plants that are present in each can.

c. **Day 8** Reexamine all of your plants carefully and record your observations. Do you think that all of the phenotypes that you assigned on Day 5 were correct? Or do you think that any of them should be changed? In which parts of the plant does it seem to be easiest to distinguish the various phenotypes? In the cotyledons? In the stems? Or in the first true leaves? Or are different traits distinguished more easily in different organs?

D.2

3. **In conclusion**

Fill out the Wisconsin Fast Plants Phase 2 Work Sheet.

D.2

Name ______________________

Date ______________ Hour __________

WISCONSIN FAST PLANTS OBSERVATIONS

Be sure to date all comments.

D.2

Name ____________________

Date ______________ Hour __________

WISCONSIN FAST PLANTS OBSERVATIONS (CONTINUED)

D.2

Name ______________________________

Date ____________________ Hour __________

WISCONSIN FAST PLANTS WORK SHEET

1. What are the two mutant traits that distinguished your PA and PB plants from one another and from wild-type Wisconsin Fast Plants?

   The PA plants are ______________________ mutants.
   The PB plants are ______________________ mutants.

2. If the mutant traits exhibited by the P_1 and P_2 generation are heritable, why didn t those two traits appear in their progeny in the F_1 generation?

   ____

   ____

3. Based on your explanation above, what would you predict that the ratio of wild-type to mutant individuals should have been for each of these two traits in the F_2 generation? Explain.

   ____

   ____

4. Above each of the tables below, record how many F_2 plants germinated and grew large enough that their phenotypes could be determined with confidence. Then in the right hand column of each table record how many of these F_2 plants had each of the indicated phenotypes.

Table 1.A. Data collected by our own group.

The number of plants analyzed was _____.

Phenotype	**Number of F_2 plants**
Wild-type with respect to the PA trait:(______________________)	
Mutant with respect to the PA trait: (______________________)	
Ratio of wild-type to mutant: __________ to __________	
Wild-type with respect to the PB trait:(______________________)	
Mutant with respect to the PB trait:(______________________)	
Ratio of wild-type to mutant: __________ to __________	

D.2

Name ______________________________

Date ____________________ Hour __________

Table 1.B. Combined data for the whole class.

The number of plants analyzed was _____.

Phenotype	Number of F_2 plants
Wild-type with respect to the PA trait:(____________________)	
Mutant with respect to the PA trait:(____________________)	
Ratio of wild-type to mutant: _________ to _________	
Wild-type with respect to the PB trait:(____________________)	
Mutant with respect to the PB trait:(____________________)	
Ratio of wild-type to mutant: _________ to _________	

5. With respect to the PA trait, how does the ratio of wild-type to mutant individuals that you predicted in Question 3 compare to the ratios of wild-type to mutant individuals that you reported in tables 1A and B?

The predicted wild-type-to-mutant ratio was ____:1

The wild-type-to-mutant ratio we observed with our group s F_2 plants was ____:1

The wild-type-to-mutant ratio observed by the entire class was ____:1

In your opinion, are these differences between the predicted and the observed wild-type-to-mutant ratios significant?

Yes ____ No ____ Can t decide ____

Explain

____

____

6. How do the predicted and observed wild-type-to-mutant ratios for the PB trait compare?

The predicted wild-type-to-mutant ratio was ____:1

The wild-type-to-mutant ratio we observed with our group s F_2 plants was ____:1

The wild-type-to-mutant ratio observed by the entire class was ____:1

In your opinion, are these differences between the predicted and the observed wild-type-to-mutant ratios significant?

Yes ____ No ____ Can t decide ____

Explain

____

____

D.2

Name ______________________________

Date ____________________ Hour __________

7. How confident are you of the validity of your answer to Question 6?
Very confident ____ Fairly confident ____ Not at all confident ____
Explain your answer:

____

____

8. Record the observed phenotypes of the F_2 plants with respect to combinations of P_1 and P_2 traits.

In tables 1A and B (Question 4) you recorded the number of F_2 plants that were wild-type or mutant with respect to the PA and PB traits individually. In the next two tables record the numbers of F_2 plants that had each of the four possible combinations of these two traits.

Table 2.A. Data collected by our own group.

The number of plants analyzed was _____.

Phenotype with respect to the PA trait: (______________)	Phenotype with respect to the PB trait: (______________)	Number of F_2 plants
Wild-type	Wild-type	
Mutant	Wild-type	
Wild-type	Mutant	
Mutant	Mutant	

Table 2.B. Combined data for the whole class.

The number of plants analyzed was _____.

Phenotype with respect to the PA trait: (______________)	Phenotype with respect to the PB trait: (______________)	Number of F_2 plants
Wild-type	Wild-type	
Mutant	Wild-type	
Wild-type	Mutant	
Mutant	Mutant	

D.2

Name ____________________

Date ______________ Hour ________

9. In the table below, compare the ratios of the four possible combinations of PA and PB traits that you and your class observed with the ratios that are predicted for this kind of dihybrid cross. In each case, set the number of double mutants to one.

Phenotypic combinations	**Predicted ratio for a dihybrid cross***	**Ratio observed in our own plants**	**Ratio observed for entire class**
Wild-type for the PA trait and wild-type for the PB trait			
Mutant for the PA trait and wild-type for the PB trait			
Wild-type for the PA trait and mutant for the PB trait			
Mutant for the PA trait and mutant for the PB trait	1	1	1

* You can obtain this ratio either by (a) using the product-of-probabilities method, (b) using a Punnett Square, or (c) reviewing similar calculations that you made for Exercise 2.C.3 (Create-a-Baby).

To obtain the numbers for each of these blanks, divide the number of plants observed in that category by the number of double-mutant plants observed in the same data set.

10. Do you think that the differences between the predicted and the observed ratios in the above table are significant?

Yes ____ No ____ Can t decide ____
Explain

____

____

11. How confident are you of the validity of you answer to Question 10?
Very confident ____ Fairly confident ____ Not at all confident ____
Explain

____

____

Note: The next unit (2.E) illustrates a mathematical method that many biologists use to determine whether differences between predicted results and observed results in an experiment such as this one are significant. You may find it interesting to review this unit, even if your teacher does not make it a class assignment.

D.2

Experimenting with Wisconsin Fast Plants: Option 2

INTRODUCTION

MOST PLANTS ARE NOT suitable for use in classroom biology projects. That is because most plants grow slowly, take months or years to produce seeds that are ready to be planted, and get so large that there is not room for more than a few of them in a classroom.

Paul Williams, a plant biologist at the University of Wisconsin, set out to change all that, and to develop plants that would be convenient and fun for students to grow and study in the classroom. To achieve his goal, he selected the most rapidly flowering plants he could find, crossed them with one another, and continued this selective breeding for several plant generations. The resulting plants are the Wisconsin Fast Plants, some of which you will use in this exercise. These plants flower in two weeks, produce mature seed in five to six weeks, and are so small that it is possible to grow hundreds of them in a classroom.

Another name for Wisconsin Fast Plants is **rapid-cycling brassicas**. *Brassica* is the genus of plants that includes cabbage, broccoli, cauliflower, turnips, mustard greens, collards, and many other popular food plants. The Fast Plants are members of the species *B. rapa*, the *Brassica* species from which bok choi and Chinese cabbage were derived.

You will use the Fast Plants to study the inheritance patterns for two visible plant traits. Organisms that are produced by a cross between genetically distinguishable parents are called **hybrids**, and when those parents differ in two heritable traits, the cross between them is called a **dihybrid cross**. You will be analyzing the results of a dihybrid cross.

Biologists have a set of terms that they use to keep track of the generations in such genetic experiments. The plants with which the experiment begins are members of the **parental**, or **P**, generation. Their offspring are called the **first filial**, or **F_1**, generation. [The Latin words for *son* and *daughter* are *filius* and *filia*, respectively.] The progeny of the F_1 generation are called the **second filial**, or **F_2**, generation.

The seeds that you will be given will produce plants representing all three generations of plants in the dihybrid cross that you are to analyze. We will call the two different types of parental plants PA and PB to distinguish them. PA and PB plants were crossed to produce the F_1 seeds. Then F_1 plants were crossed to produce the F_2 seed. You will also be given some double-mutant seeds, so that you will be able to see what plants will look like if they have a combination of the mutant traits of the PA and PB plants.

D.2

The objective of this experiment is to determine what sorts of visible traits that were present in the P-generation plants disappear in the F_1 generation, but then reappear in the F_2 generation, and in what combinations they reappear. Because you may want to use a mathematical method to test certain genetic hypotheses, it will be important to germinate a substantial number of F_2 seeds, and to keep careful track of how many plants of each different phenotype you see.

MATERIALS

For the class:
- 1 container of potting mix
- 2 spoons
- 1 ruler
- 1 bottle of 1/8 X Peter s Professional Fertilizer

For each group of four students:
- 1 film-can growth system
- 1 water bottle
- 1 envelope containing 6 PA seeds
- 1 envelope containing 6 PB seeds
- 1 envelope containing 6 F_1 seeds
- 1 envelope containing 18 F_2 seeds
- 1 envelope containing 6 double-mutant seeds

For each set of four groups of four students:
- 1 plant lighthouse
- 1 piece of foil-covered cardboard
- 6 pieces of 1 inch thick insulating foam

D.2

PROCEDURE

You will grow your plants in a film-can growth system that consists of several parts. The film cans (Fig. 1) will hold your plants and the soil in which they grow; The other pieces form an automatic watering system that will keep the soil moist and give your plants a constant supply of water and nutrients.

Put a piece of masking tape on the bottom chamber of your growth system, and with the marking pen write your group name and your class period. (Make sure that the tape does not overlap the clear stripe on the bottom chamber, because you will need to look through this stripe to monitor the fluid level.)

1. **Preparing the growth system and planting the seeds (Day 1)**

    a. Make sure that a wick is about half in and half out of each can (Fig. 1).

    b. Hold your film-can cluster over the container of soil and fill each can loosely. Tap the film cans on the side to help the soil settle, but DO NOT PRESS THE SOIL INTO THE CANS.

    c. Use the ruler to scrape off any excess soil so that each can is filled up to but not over the top (Fig. 2).

    d. Make sure that both ends of the long wick hang out of your slotted tray so that they will hang freely into the nutrient reservoir (Figs. 3-4).

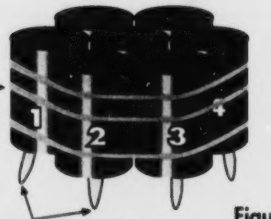

Film-can cluster held together with rubber bands
1
2
3
4
Wicks half in and half out of each film can

Figure 1

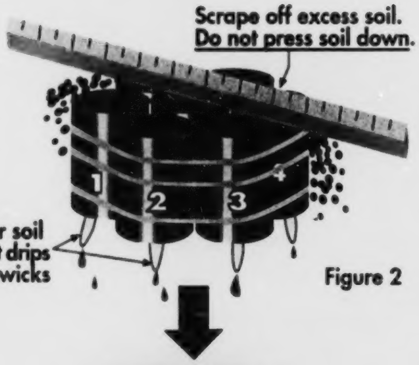

Scrape off excess soil. Do not press soil down.
1
2
3
4
Water soil until it drips from wicks

Figure 2

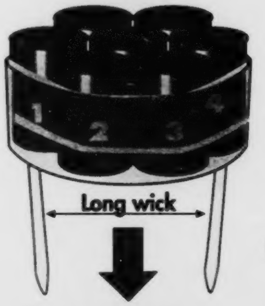

1
2
3
4
Long wick

Figure 3

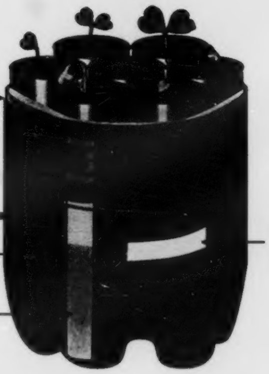

Slotted tray with Film-can cluster
Nutrient level
Wick
Nutrient level viewing window
Tape with group name

Figure 4

e. Hold the film-can cluster over the slotted tray. Turn your water bottle upside down and squeeze gently to direct a stream of water at each film can. Water each can until water drips from its wick (Fig. 2). (This will cause the soil to settle somewhat.)

f. Water the wick in the slotted tray until it is saturated and water runs freely into the nutrient reservoir.

g. Place the film-can cluster in the slotted tray. Paying attention to the numbers on your film cans, place six evenly spaced seeds on the surface of the soil in each film can, according to the following plan:

   Can 1: six PA seeds.
   Can 2: six PB seeds.
   Can 3: six F_1 seeds.
   Cans 4-6: six F_2 seeds each.
   Can 7: six double-mutant seeds.

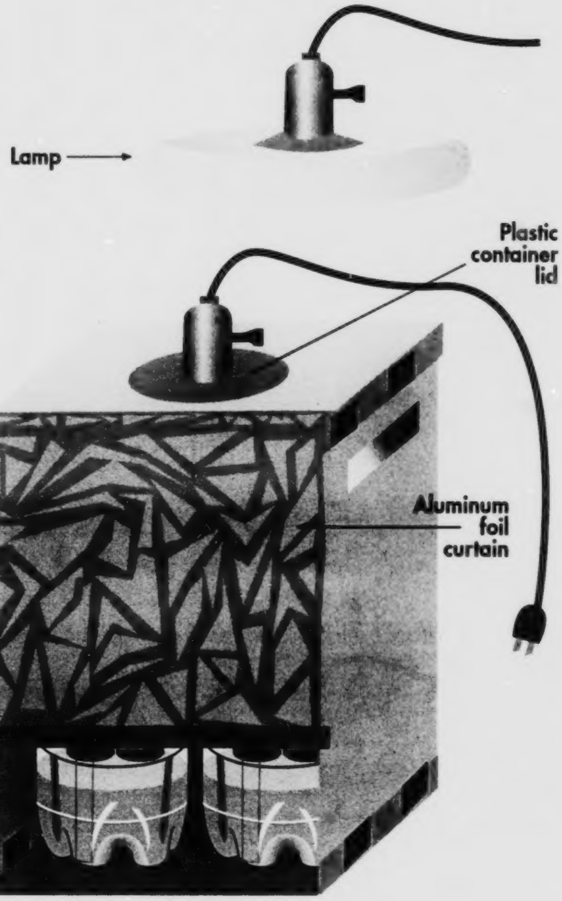


Figure 5

h. Take your film-can cluster back to the container of potting mix. Carefully sprinkle soil over the seeds to fill each can to the top. Level the soil with the ruler. DO NOT PRESS THE SOIL INTO THE CANS.

i. Add 1/8 X Peter s nutrient solution to the nutrient reservoir until the level is just below the bottom of the slotted tray.

j. Place your film-can cluster in the slotted tray. Water each can gently until water begins to run out of it and through the slots into the nutrient reservoir.

k. Your plants will be grown in a plant lighthouse (Fig. 5). Take your growth system to the plant lighthouse assigned to you by your teacher.

l. If the following has not already been done, stack six foam blocks on the floor of the lighthouse and then place the foil-covered cardboard on top of the stack.

m. Place your growth system on top of the foil-covered cardboard, toward one corner of the lighthouse.

2. **After Day 1**

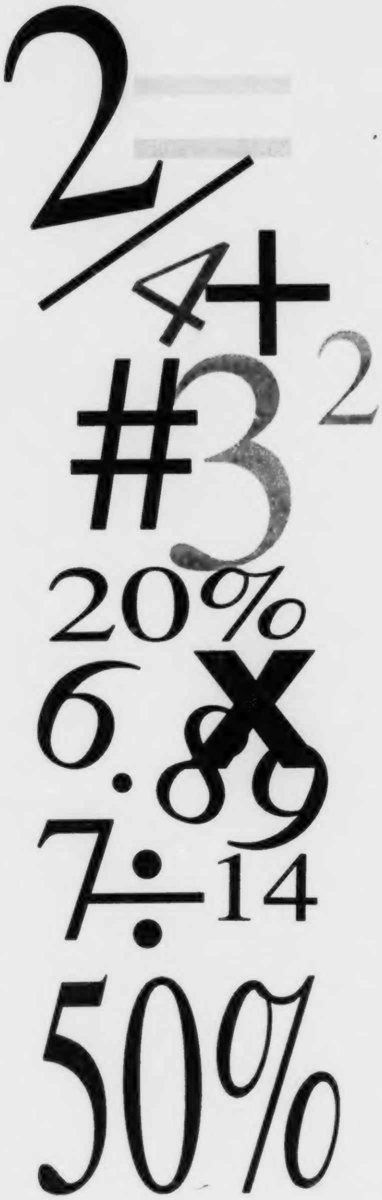
a. **Days 2-4** Use your water bottle to sprinkle the surface of the soil in each film can.

b. **Days 3-5** Observe your film cans daily and record what you see on the Observations pages. Record how many seedlings emerge in each can and when they emerge. Each day after that observe all seedlings carefully, looking for significant differences among them. Compare your plants to the photographs of mutant Fast Plants or to the live Fast Plant mutants that your teacher put on display. By Day 5, assign phenotypes to all the plants that are present in each can.

c. **Day 8** Reexamine all of your plants carefully and record your observations. Do you think that all of the phenotypes that you assigned on Day 5 were correct? Or do you think any of them should be changed? In which parts of the plant does it seem to be easiest to distinguish the various phenotypes? In the cotyledons? In the stems? Or in the first true leaves? Or are different traits distinguished more easily in different organs?

3. **In conclusion**

Fill out the Wisconsin Fast Plants Work Sheet.



CHAPTER 2

Passing Traits from One Generation to the Next

SECTION E

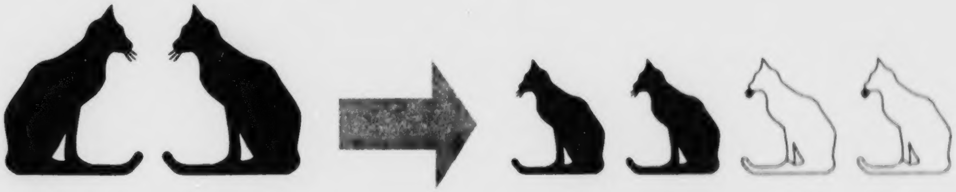
How Are Genetic Results Evaluated Statistically?

E.1

Introduction to Using Statistics to Evaluate Genetic Explanations

GENETICS IS ALL ABOUT using the seen (the visible phenotype) to infer the unseen (the invisible genotype). Sometimes, however, math can be used to determine whether or not the inference that you have drawn about the genotype-phenotype relationship is a reasonable one.

As an example of how this works, let's suppose that you have a pair of black cats at home that mated and produced a litter of two black kittens and two white ones. How would you explain this?



Well, because you've just learned all about dominant and recessive alleles while you were studying Reebops, Make-A-Babies, and red and white yeast cells, you'd probably suggest that both Momcat and Dadcat must be heterozygous with respect to coat-color alleles, each having one dominant "black" allele and one recessive "white" allele, and that kittens that happened to receive one white allele from each parent developed the white phenotype. In short, you'd use the visible phenotypes of the cats and kittens, together with your understanding of possible genotype-phenotype relationships, to make a reasonable inference about the genotypes of both the parent cats and the kittens.

REAL COOL, BABY!

However, when you show your kittens to your friend Katy, and tell her how you explain their coat colors, she disagrees strongly. She says, "Based on what we learned in class, if Momcat and Dadcat really were heterozygous for a pair of black/white coat-color alleles, they should have had three black kittens and one white one, not two of each!" You think about that for a minute and respond, "Well, 2-to-2 is not that far from 3-to-1, and the difference could be due just to chance. After all, Katy, you know you won't always get two heads and two tails if you flip a coin four times." Katy says, "Well that may be, but 1/2 white kittens doesn't seem to me to be very close to the 1/4 white kittens that your hypothesis predicts." After arguing about this for a while longer, you finally agree to discuss it with your teacher.

E.1

After you explain the problem to your teacher, he says, "The way to settle this is not by arguing about it, but by analyzing your results statistically to see whether Katy is correct in thinking that you need to think about some alternative explanation for the kittens. I recommend running a Chi square test, because Chi square is specifically designed to determine whether or not the difference between the results you observed and the results that were predicted by your genetic hypothesis are too great to be due to chance alone."

E.2

Too Many White Kittens? Using Chi Square (χ^2) to Find Out

THE INSTRUCTIONS YOUR TEACHER gave you for running a Chi square test on your black/white kitten data were as follows:

- The formula for determining the value of χ^2 (Chi square) is:

$$\chi^2 = \frac{(\text{Observed - Expected})^2}{\text{Expected}} \textbf{ summed for all classes*}$$

* In this formula the term "summed for all classes" refers to all classes of objects that were observed, not all classes of students who made such observations!

- The way you would apply this formula to your kittens, which fall into two classes (black and white) is:

$$\chi^2 = \frac{(\text{Obs. black - Exp. black})^2}{\text{Exp. black}} + \frac{(\text{Obs. white - Exp. white})^2}{\text{Exp. white}}$$

or, more specifically (since your hypothesis predicts that 3/4 of the kittens should be black and 1/4 should be white):

$$\chi^2 = \frac{(2-3)^2}{3} + \frac{(2-1)^2}{1} = \frac{1}{3} + \frac{1}{1} = 1.33$$

- Your χ^2 value is 1.33.
- To find out the meaning of this χ^2 value, you must look it up in a standard table like the one below. But before you can do this, you must decide how many **degrees of freedom** you have, so you will know which line to look in.
- The degrees of freedom in such an experiment is one less than the number of classes you distinguished. Since you distinguished two classes in this case (black and white kittens) you have one degree of freedom.

	p (The probability that the difference seen between the observed and the expected values could be due to chance alone.)								
Degrees of Freedom	**90%**	**70%**	**50%**	**30%**	**20%**	**10%**	**5%**	**1%**	**0.5%**
1	0.02	0.15	0.46	1.1	1.6	2.7	3.8	6.6	7.9
2	0.21	0.71	1.4	2.4	3.2	4.6	6.0	9.2	10.6
3	0.58	1.4	2.4	3.7	4.6	6.2	7.8	11.3	12.8

- When you search along the '1 degree of freedom' line, you find that a χ^2 value of 1.33 lies somewhere between the 20% and the 30% columns. This means that if the hypothesis on which you based your predictions is correct, the difference you observed between the predicted numbers and the actual numbers of black and white kittens can be expected to occur (in a litter of four) more than 20% of the time, due to chance alone.

By convention, scientists usually use a 5% cut-off value to decide whether or not to reject the hypothesis on which a set of predicted values is based. More specifically, if deviations as large as those observed could be expected to occur more than 5% of the time due to chance alone, then the deviations are not large enough to justify rejecting the hypothesis on which the predictions were based.

- What this result does not mean: The results of the Chi square test you just performed does not prove that your hypothesis about the inheritance of these coat colors is correct, only that it is possibly correct. [The results of a Chi square test can never prove that a hypothesis is correct, although they can indicate that a hypothesis is very unlikely to be correct.]
- What your Chi square result does mean, however, is that Katy's rejection of your hypothesis, based solely on the difference between the observed and expected number of black and white kittens in your litter, was not scientifically sound.

AN IMPORTANT POINT: A Chi square test is very sensitive to the size of the sample observed. Thus, although a black kitten:white kitten ratio of 2:2, instead of 3:1, is not adequate to cause rejection of your genetic hypothesis when only one litter of four kittens was observed, it would have been a very different matter if the same 2:2 ratio had been obtained after examining several litters containing a total of 40 kittens. We can see this very easily by calculating χ^2 for such a sample size:

$$\chi^2 = \frac{(20 - 30)^2}{30} + \frac{(20-10)^2}{10} = \frac{100}{30} + \frac{100}{10} = 13.3$$

If we look up this χ^2 value of 13.3 on the '1 degree of freedom' line, we find that it indicates that this large a deviation from the predicted 3:1 ratio would be expected to occur by chance much less than 0.5% of the time. This is a very different outcome than we obtained when we saw the same ratio of black to white kittens in a single litter of four kittens, and it would clearly indicate that your original hypothesis to explain black versus white coat color in kittens should be rejected, and a new hypothesis should be sought.

- Because χ^2 is so sensitive to sample size, it must always be calculated using the actual numbers observed, and never using fractions, decimal fractions or percentages instead of actual numbers!

E.3

How to Perform a Chi-Square Test on Any Data Set

THE PROCEDURE USED TO perform a Chi square test on the black/white kitten data can be generalized to analyze the results of any genetic experiment as follows:

STEP 1. State a simple genetic hypothesis that makes a precise prediction about how the offspring resulting from some particular mating should be distributed between various phenotypic classes.

[In the case of the kittens, the hypothesis was that Momcat and Dadcat were both heterozygous with respect to a pair of alleles that control coat color: the dominant black allele and the recessive white allele. The prediction made from this hypothesis is that Momcat and Dadcat should have three black kittens for every white one.]

STEP 2. Count the actual offspring to determine how they actually are distributed between those phenotypic classes.

[In the case of the kittens this was very simple: there were two black and two white kittens.]

STEP 3. Determine the number of offspring that are to be expected in each phenotypic class. To do this, multiple the total number of offspring that were actually produced by the frequency with which each phenotypic class is expected to occur.

When we did this for the kittens it was misleadingly simple, because there were exactly four kittens in the litter, and the two phenotypic classes (black and white) were expected with frequencies of 0.75 and 0.25, respectively. Thus when we multiplied 4 x 0.75 and 4 x 0.25, we got integral numbers: 3 and 1, respectively.

But suppose there had been five in the litter of kittens Momcat gave birth to. In this case the expected number of black kittens would have been 5 x 0.75, or 3.75, and the expected number of white kittens would have been 5 x 0.25, or 1.25. Yes, you may – and indeed in most cases you will – get non-integral numbers of individuals expected in each class.

E.3

STEP 4. Calculate χ^2 according to the formula:

$$\chi^2 = \frac{(\text{Observed - Expected})^2}{\text{Expected}} \text{ summed for all classes}$$

STEP 5. Estimate your p value using the χ^2 table like the one shown in E.2. (This is a standardized table of values that can be found in all sorts of scientific textbooks and reference books.) Remember that the number of degrees of freedom you will use will be one less than the number of phenotypic classes predicted by your hypothesis.

STEP 6. Use the 5% rule to decide whether or not your data are consistent with your genetic hypothesis. That is, unless your χ^2 value is smaller than the one given in the 5% column (indicating that deviations from expected values as large as the ones that you obtained could occur more than 5% of the time by chance alone), you should search for a genetic hypothesis that is more consistent with your data.

Now let's use the chi square method to analyze your Wisconsin Fast Plant data.

E.3

Name ______________________________

Date ____________________ Hour __________

WISCONSIN FAST PLANTS CHI SQUARE WORK SHEET B

The data necessary for performing the following Chi square tests should be recorded on your Fast Plants Phase 2 Work Sheet.

Part One: A Simple Trial Run

To become familiar with running the Chi square test, let's apply it to just one of the mutant traits that showed up in your F_2 plants.

One half of the class should calculate χ^2 and p values for the trait that was exhibited by the PA plants, and the other half of the class should do this for the PB trait. In both cases the pooled data from the entire class should be used for the analysis. And in both cases we will assume that the hypothesis to be tested is that the trait in question is controlled by a single gene with two alleles, with the mutant allele being recessive to the wild-type allele. Thus, in both cases the expected wild-type-to-mutant frequency will be 3:1.

The mutant trait I am analyzing by χ^2 is ______________________

Total number of F_2 plants obtained by the class: ________.

Phenotype	**Expected #**	**Observed #**	**Difference**	**(Difference)^2**
Wild-type				
Mutant				

Multiply the total number of F_2 plants by the expected frequencies (3/4 and 1/4) to get the expected numbers of plants in each category. You probably will get non-integral numbers.

$$\chi^2 = \frac{(\text{Observed - Expected})^2}{\text{Expected}} \text{ summed for all classes}$$

(Remember: in this formula "classes" refers to phenotypic classes of plants, not classes of students.)

χ^2: ________ p__________ Use the table on page S150 to determine the value of p.

Conclusion: ____

____

E.3

Name ______________________________

Date ____________________ Hour ____________

Part Two: Chi Square Analysis of the Two Phenotypes at Once

Now that you have performed a Chi square analysis for one phenotype (the PA or PB trait), it should be easy for you to modify the analysis to consider both phenotypes at once.

What you will be able to test this way is whether plants with the four possible combinations of mutant and wild-type traits are present in the F_2 generation in proportions that are not significantly different from the proportions that are predicted by either a Punnett Square diagram, or a product-of-probabilities calculation. If the observed ratios are not significantly different from the predicted ratios, this will indicate that alleles at the two different loci that are being considered exhibit what biologists call "independent assortment." (That is to say, the allele an offspring receives from one of its parents at one locus is independent of the allele that it receives from that parent at the other locus.)

Independent assortment of alleles at two loci always occurs if those loci are on separate chromosomes. However, if the two loci are located close together on the same chromosome, they will exhibit "linkage," which is the opposite of independent assortment. In such cases, an allele at one locus will travel from parent to offspring together with the allele at the second locus with which it is physically linked on a particular parental chromosome. This will result in the F_2 generation exhibiting an overabundance of individuals with the two allelic combinations that their grandparents had, and a deficiency of the other two possible combinations. In extreme cases of linkage, only two of the four possible combinations will be observed.

So, in short, what we will be asking with this next Chi square analysis is whether or not the relative abundance of F_2 plants with the four different phenotypic combinations is significantly different from the proportions predicted on the assumption of independent assortment of alleles at the two different loci we are studying.

COMBINED DATA FOR THE ENTIRE CLASS **TOTAL NUMBER OF F2 PLANTS: _____**

PA phenotype	PB phenotype	Frequency expected*	Number expected	Number observed	Difference	Difference^2
Wild-type	Wild-type					
Mutant	Wild-type					
Wild-type	Mutant					
Mutant	Mutant					

*You can obtain these frequencies by either (a) using the product-of-probabilities method, (b) using a Punnett Square, or (c) reviewing similar calculations you made for previous exercises.

χ^2: ________ p__________

E.3

Name ______________________________

Date ____________________ Hour __________

Conclusion: ______________________________

____

CHAPTER 2

Passing Traits from One Generation to the Next

CHAPTER 2

Passing Traits from One Generation to the Next

CHAPTER TWO OVERVIEW T100

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3. How to Perform a Chi-Square Test on Any Data Set .... S152 ..... T170

Chapter 2 Overview

IN CHAPTER 1, YOUR students were introduced to the concept that DNA is the carrier of hereditary information. This should have raised more questions in their minds than it answered. For example, how does DNA get passed on from parents to children? Or if each child inherits DNA molecules from both parents, doesn't this mean that each person must have twice as much DNA as his or her parents? Or if my sister, my brother, and I all got our DNA from our Mom and Dad, and if the DNA we inherited determines all of our heritable traits, why don't we look just like one another? And why don't we look like some sort of average of Mom and Dad?

The purpose of this chapter is to help students answer such questions. To do this, we must move the discussion of heredity to a slightly more complex level. We have to consider how DNA actually gets passed on between the generations. And we have to think about how the information that this DNA contains is used to generate a whole new individual, not just a bunch of new protein molecules.

CHAPTER 2

Passing Traits from One Generation to the Next

SECTION A

What is Inheritance?

A.1

An Introduction to Inheritance

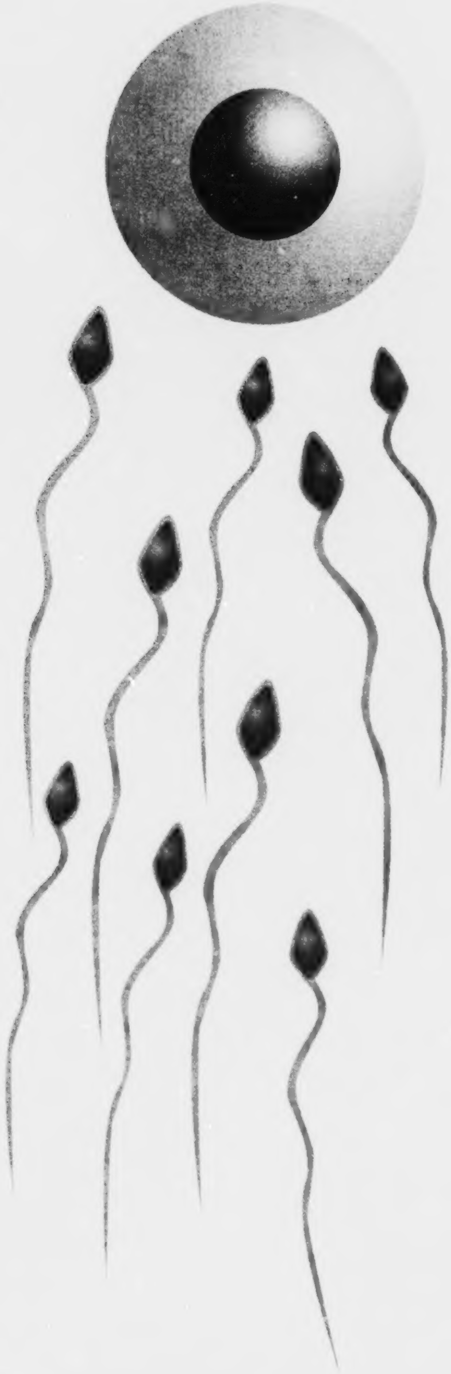
STUDENT PAGES 76-77

LESSON OVERVIEW

This section consists of only one part: a reading assignment designed to familiarize your students with the general concepts underlying the study of inheritance.

TIMELINE

It will take an average student 15 to 20 minutes to read this material. It could be assigned as homework.



CHAPTER 2

Passing Traits from One Generation to the Next

SECTION B

How Does a New Generation Get Started?

Chapter 2: Section B Background

IT MIGHT SEEM THAT studies of embryos are out of place in a discussion of genetics. But nothing could be further from the truth.

It is only through the processes of embryonic development that the potential present in the DNA in an egg and a sperm can be converted into a physical reality – a new individual. Or, to say the same thing in technical terms that your students will learn to use in this chapter, it is during embryonic development that a **genotype** (an individual's complete set of genes) becomes transformed into its **phenotype** (its set of visible features).

This section begins with a discussion of what **model systems** are, and how they are used both to study heredity and development, and to yield insight about humans that could never be obtained by studying human beings directly.

In the second part of this section, students will work with one such model system, sea urchins, which are the premier model for studying the first few stages of the beautiful and mysterious process by which genotype is transformed into phenotype. Here they will watch the fusion of egg and sperm and the initial stages of embryonic development.

In the third and final part of this section, they will encounter an even more captivating example of genotype-to-phenotype transformation. In the *Nova* video, *The Miracle of Life*, they will benefit from extraordinary, award-winning cinemagraphic techniques that record the development of a human being from the time when egg and sperm are released, through the instant of their fusion, and the subsequent nine months of development, to the moment of birth.

B.1

Model Systems for Studying Heredity & Development

STUDENT PAGES 80-81

LESSON OVERVIEW

Progress in genetics has always been most rapid when geneticists have found an appropriate **model system,** an organism whose biological features make it very well suited for the experimental analysis of a specific set of questions.

Garden peas were the ideal model system for Gregor Mendel, a monk and botanist who was interested in asking the basic questions about heredity 150 years ago. But garden peas could never have been used to determine the chemical nature of the hereditary material or the way it works. For addressing that sort of genetic question, bacteria turned out to be the ideal model. But bacteria are of little value in addressing questions about the way particular genes control the development of specific body parts in complex organisms like ourselves. Such questions must be addressed with animals. The animals that have been most useful as model systems for asking this kind of developmental-genetic question include fruit flies, round worms, zebra fish, frogs, and mice.

Even the most ardent advocates of such developmental-genetic models have regularly been quite astonished, however, to discover the extent to which studies of the genetic control of embryonic development in one of these model systems turns out to apply to others – including ourselves. An expanded version of the example that is mentioned in the student pages may help to underscore this very important concept.

A few years ago, biologists studying fruit fly development discovered that an eyeless fly was produced whenever both copies of one particular gene were defective. For obvious reasons, they named this the "eyeless" (*eye*) gene. The wild-type *eye* gene is normally expressed (transcribed and translated) only in the region of the fruit fly head where the eyes will form. But when cells on a leg, or the back, or a wing of the fly were tricked into expressing the wild-type *eye* gene and making the corresponding protein, extra eyes developed in those regions of the leg, or back, or wing! This clearly suggested that the *eye* gene is a "master control gene" that is responsible for triggering the entire series of events that is involved in making an eye. Soon it was found that all animals with eyes, including squid, sea scallops, flatworms, fish, birds, mice, and humans, have a gene of extremely similar DNA sequence that plays a similar role in eye development in every case. In fact, the mouse version of the *eye* gene is so similar to the fly gene, that when the mouse gene was introduced into fruit flies and expressed anywhere on the fly body, an extra eye was formed in that location. (Notably, the eyes that formed under these conditions were fly eyes, not mouse eyes.)

Human babies who have the misfortune to be born with one defective copy of the human version of the *eye* gene have small eyes that lack an iris, which results in serious vision problems. Babies that are born with two defective copies of this gene have no eyes at all. Thus, it is clear that the *eye* gene plays the same role in human eye development as its counterpart does in that of the fly.

Similarly detailed genetic parallels between flies, birds, mammals and ourselves have been discovered with respect to the **master control genes** that control development of the heart (a gene called *tinman*), legs, nervous system, and other body parts. If most biologists had not already been thoroughly convinced that (as a famous biologist said 50 years ago), "nothing in biology makes sense except in the light of evolution," such modern observations would undoubtedly drive them to such a conclusion.

TIMELINE

It will take an average student 10 minutes to read this material; it could be assigned as homework.

REFERENCE

The Genes We Share with Yeast, Flies, Worms and Mice: New Clues to Human Health and Disease. (2001). Available by writing to the Howard Hughes Medical Institute, Office of Communications, 4000 Jones Bridge Road, Chevy Chase, MD 20815-6789. This colorful and well-written booklet is the eighth in a series of reports about biomedical research that has been prepared by the Howard Hughes Medical Institute specifically to provide teachers and students with up-to-date information about current research developments in biology and biomedical sciences. It is an unusually rich source of information about the powerful new methods that are now being used to study the genes of model organisms as well as the novel insights that such studies have already provided us about our own genetic makeup.

B.2

Starting a New Generation: Sea Urchin Fertilization

STUDENT PAGES 82-85

LESSON OVERVIEW

This activity is meant to bring the processes of early development vividly to life for your students. The sea urchin sperm and eggs that they will see under their microscope serve as wonderful models to help them understand their own biological and genetic origins. Students enjoy working with live animals, particularly ones that are unfamiliar, yet non-threatening. So this is a wonderful time to talk about the unity and diversity of life: It is the fundamental unity of all life forms that allows a model organism such as the sea urchin to give us insights into our own biological nature. This exercise is also, of course, a wonderful time to introduce or review the concepts of mitosis and meiosis, and the roles that each plays in the processes of heredity and development.

TIMELINE

The sea urchins need to be ordered at least two weeks before you plan to use them in your class. They are only shipped on Monday or Tuesday and will usually arrive sometime during the next day. Be sure you are prepared to take care of the urchins properly when they arrive (see below). It is best to do the fertilization exercise as soon as possible after delivery. So plan accordingly.

MATERIALS

For the entire exercise:
3 or 4 aquariums or holding tanks (see Advance Preparation for details)
1 package of a dry artificial sea water mixture (such as Instant Ocean™)*
1 hydrometer*
1 aquarium pump*
3 or 4 air stones*
tubing and adapters to connect air pump to air stones*
sea urchins
2-5 ml syringe with a 20-30 gauge needle
30 ml of 0.5 M KCl (1.13 g of KCl in 30 ml water)
disposable petri dishes
dropping pipettes
microscope slides and cover glasses for checking gametes
1 or 2 microcentrifuge tubes for storing sperm
3 or 4 250 ml beakers to hold female sea urchins during spawning
3 or 4 16 x 100 mm test tubes for making sperm suspensions
3 or 4 50 or 100 ml beakers for fertilizing eggs
1 500 ml bottle for cultivating embryos

*available at most pet stores, or from lab supply catalogs

For each group of four students:
- 1 or 2 depression slides
- 2 or 3 dropping pipettes
- a compound microscope

Sea urchins can be ordered from:
Carolina Biological Supply
(800) 334-5551
www.carolina.com
Sea Urchin Embryological Kit, catalog no. BA-16-2505 or
Sea Urchins, catalog no. BA-216-2949

Pacific Bio-Marine Laboratories
(310) 677-1056

Gulf Specimen Corp.
(904) 984-5297

Urchins are sold by Carolina Biological Supply Co. in sets of 14, which is usually adequate to supply enough gametes for all of the classes taught by one (or possibly even two) teachers. Because different species are available from different suppliers and/or at different times of the year, and because this will affect the way you will need to handle the urchins when they arrive (as explained below), it is a good idea to ask what species will be shipped when you place your order.

ADVANCE PREPARATION

- Order sea urchins at least three weeks before the activity.
- Visit the sea urchin website (http://www.stanford.edu/group/Urchin/fert.htm) well before the urchins arrive. As discussed in the reference section below, this site is full of useful and interesting information.
- Have 3-4 aquaria or holding tanks filled with sea water, aerated, and equilibrated for a few days by the time the sea urchins are scheduled to arrive. See discussion of Sea Urchin Care below to determine temperature at which your aquaria or holding tanks should be equilibrated.
- If you have aquaria already on hand, use them. However, genuine aquaria are not essential. Large, clear plastic boxes (such as 3-gallon Rubber Maid, #2220) purchased at a discount store will work equally well as holding tanks. You should soak them for a few days with several changes of tap water (to leach out any plasticizers that might be toxic to sea urchins) before filling with sea water.
- Air pumps, air stones, a hydrometer, and sea salt are available in many pet stores. These supplies can be obtained from most any biological supply company as well.
- Prepare the sea water according to the instructions on the package, using dechlorinated tap water. Check the specific gravity with the hydrometer. Adjust as necessary to a spe-

cific gravity of 1.020 and 1.023 (at 75°C), adding more sea salt to increase or more dechlorinated water to decrease the specific gravity.

- Collect and organize other supplies. Most of the additional supplies can be purchased from any scientific supply company. For example, from Fisher Scientific [(800) 766-7000, www.fishersci.com] you might order:

  Potassium chloride, 500 g, cat. no. P217-500
  Plastic dropping pipettes, box of 400, cat. no. 13-711-37
  Hanging drop depression slides, package of 12, cat. no. 12-560A
  3 ml disposable syringes with 22 Ga needles, box of 100, cat. no. 14-826-85

- It is important to use glassware and other supplies that are clean and free of detergent residues or other potentially toxic substances. Glassware that has been used previously should be rinsed extensively with clear tap water before being used for this exercise.
- When you finish this exercise, rinse all of your equipment thoroughly with clear water. Do not use any soap or detergent, or you may leave a residue on them that will kill next year's sea urchins.

SEA URCHIN CARE

Identify which species of urchins you received. Different species have different fertile seasons, so the month in which you order will often determine which species you will get. The four species most commonly shipped from the sources listed above are:

Strongylocentrotus drohbachiensis, a cold-water North Atlantic species;
Strongylocentrotus purpuratus, a purple, cold-water Pacific species;
Lytechinus variegatus, a light-colored, warm-water species from Florida;
Arbacia punctulata, a dark purple, warm-water species from Florida.

If you have received either of the warm-water species, they should be kept in tanks or holding trays in a cool room (60-68° F) but not a refrigerator. Your prospects of having healthy, cooperative sea urchins for your class will be improved if you can allow the urchins time to become acclimated to their new environment gradually. If they were shipped in bags of sea water, float the bags in the aquarium about half an hour. Meanwhile, assess each urchin. Examine its spines and the water it has been shipped in. If its spines are falling off, or it smells foul when the bag is opened, it is dead or dying; discard it. If the urchin looks healthy, and its water smells all right but is cloudy, it is likely that the urchin has spawned. If you put an urchin that has spawned in a tank with ones that have not, chemicals diffusing from the eggs or sperm will usually cause the others to spawn immediately, ruining them for your purposes. So place any questionable urchins in individual beakers or jars of sea water, not in an aquarium with other urchins.

Once the bags with healthy urchins have equilibrated in temperature with the aquarium, open the bags and use the hydrometer to check for salinity differences between the aquarium and the sea water in the bag. If there is a difference, add portions of the aquarium water to the bag. When you have doubled the volume of the water in the shipping bags, remove the sea urchins from the bags and place them in the tank. One sea urchin per half gallon of sea water is a good ratio.

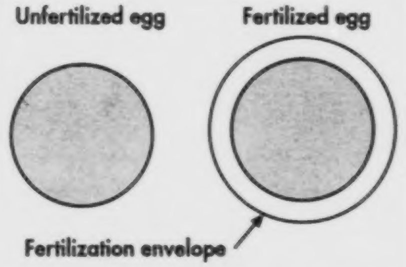
Sometimes sea urchins are shipped in sea-water-soaked, shredded newspaper rather than in bags of water. If this is how your urchins have arrived, unwrap each one carefully and examine it. If its spines are falling off, or it smells foul, it is dead or dying and should be discarded. If you see a white or yellow-orange exudate on the **test,** or shell, between the spines, this will indicate that the urchin has spawned; isolate it from the rest of the urchins, and rinse your hands thoroughly before handling other urchins. Place healthy looking urchins in individual beakers or bags of cool (not cold) sea water, float the bags in the aquarium, and monitor carefully for the next half hour or so. If the water in a bag turns cloudy during this period, this also will indicate that the urchin has spawned and should not be housed with unspawned ones. Place healthy, unspawned urchins in a tank together, as above.

If you have received either of the cold water sea urchins, either use them immediately or keep them in refrigerated sea water. Assess each urchin as above. Place the healthiest looking ones together in a shallow plastic tray with enough sea water to just cover them. Place them in a refrigerator at 45-50° F. Urchins held in this manner should remain healthy for 1-2 days.

PROCEDURE FOR COLLECTING AND USING SEA URCHIN GAMETES

Experience suggests that the chances of success are substantially increased when the teacher injects the urchins and collects the gametes rather than charging the students to do it. However, it is a good idea to allow students who wish to do so to hold and examine one of the urchins. (For many of them it will be a novel and broadening experience.)

1. Fill a syringe with 2.0 ml of the 0.5M KCl solution.
2. Hold a sea urchin mouth side up. Insert the needle through the soft membrane surrounding the mouth, while pointing the needle away from the mouth. There may be a little initial resistance, but then the needle should slide in easily. *Slowly* inject the KCl solution.
3. Following injection, place the urchin, mouth down, on a clean, dry Petri dish. Check periodically for appearance of an exudate (the gametes) between the spines on the upper part of the test. It may take 5-10 minutes for the gametes to appear, and some urchins may not be mature and will not release gametes in response to the KCl.
4. If you see a smooth, milky white fluid being released, you have a male that is releasing sperm. But if you see a somewhat grainy suspension being released that is colored yellow to red (depending on the species), you have a female releasing eggs. If in doubt as to which kind of gametes are appearing, use a pipette to put a small drop on a microscope slide, dilute it with a drop of sea water, and examine it under the microscope.
5. Immediately after you have determined the sex of your sea urchin, do one of the following: Female urchin: Place her, mouth up, over a 250 ml beaker that contains enough sea water to make contact with the test. Allow the eggs to flow to the bottom of the beaker. Male urchin: Leave him, mouth down, on a dry Petri dish. Use a dropping pipette to pick up the sperm from the surface of the test and transfer them to a dry microcentrifuge tube. As long as the sperm have not come in contact with sea water, they will remain alive in such a tube for at least 24 hours in a refrigerator (45° F).



A sure sign that an egg has been fertilized is the presence of a fertilization envelope surrounding the egg at a distance. (Before an egg is fertilized, this transparent fibrous structure is attached to the surface of the egg, where it is invisible. But within seconds after sperm-egg fusion it swells, moves away from the surface, and becomes visible. Its function is to prevent a second sperm from reaching the egg.)

6. As soon as you have a reasonable number of eggs in the bottom of a beaker, check the appearance of a drop of egg suspension with a microscope. If there is a lot of debris visible around the eggs, wash them. To wash the eggs, transfer the female to a second beaker of sea water to continue spawning. After the eggs in the first beaker have settled completely, pour off the water and add fresh sea water. Repeat the process. A clean suspension of healthy eggs will survive in a refrigerator for several hours.
7. As soon as you have a clean suspension of eggs, have each group of students transfer a small sample to a depression slide, examine it in the microscope, and draw what they see. Emphasize that their best chances of seeing fertilization take place will occur if they have only 10-20 eggs in the depression slide, and that if they have more, they should remove some of them and replace them with more sea water.
8. After all students have eggs on their slides, the sperm can be prepared. Dilute one or two drops of the "dry" sperm in 10 ml of sea water. Use a microscope to view the sperm with a 40X objective to make sure that they are moving. The sperm are only viable for about 15 minutes after they have been exposed to sea water.
9. As soon as you have an active sperm suspension, transfer two or three pipettefuls of an egg suspension to each of two clean small beakers and add a pipetteful of sperm suspension to each. Label each beaker with the time that the sperm and eggs were mixed.
10. Now have each group of students add a drop of the sperm suspension to the eggs in their depression slides. Emphasize that they should only need a little of the sperm suspension, enough so that they will see 10-100 sperm around each egg.
11. Check samples of the sperm-egg mixtures you have in the beakers to be sure that the eggs have been fertilized (see drawing). If they have, have each group of students examine a drop of the suspension every 15 minutes or so to monitor development. The first cleavage division should occur about an hour after fertilization.
12. Place the remaining unfertilized eggs in the refrigerator for use later in the day. Save samples of fertilized eggs from one class to the next, so students can see the later stages of development.
13. Toward the end of the day, transfer a suspension of developing embryos to a clean, dry, screw-cap 500 ml bottle and dilute it to about 100 ml with sea water. Screw the cap on tightly and float the bottle on its side in the aquarium. Samples of this preparation can then be examined over the next day or two to monitor development and behavior of the sea urchin larvae or **plutei** (singular **pluteus**).

HINTS AND TROUBLESHOOTING

1. If your schedule permits, it would be a good idea to fertilize a batch of eggs one to two hours before your first class, so that students in that class will be able to observe some cleaving embryos. Alternatively – or in addition – you may want to make arrangements so that students can drop in later in the day to observe more advanced stages of development.
2. If you have a video microscope, have it available for this exercise, so that the whole class can observe any particularly nice specimens at the same time. Also, try to use a VCR to record any particularly good views of fertilization and cell division for later viewing.
3. One never knows for sure what is going to happen when working with live animals. It is best to be prepared for the unexpected. It is possible that in the first hour you could inject five urchins and they would all be males (or all females). The thing to remember is that once you get one female and one male, you should have enough gametes to last for all your classes that day. Many teachers have backup activities planned for down time and in case the urchins are uncooperative. Some ideas include: a videodisc or tape with sections on mitosis, meiosis, and fertilization, a video from other classes where the activity was successful or prepared microscope slides of sea urchin development. If computers with Internet access are available, students can view the sea urchin website referenced above.
4. If you get eggs and sperm, but the eggs do not seem to be getting fertilized, check the temperature of the sea water and make certain that they are not getting overheated. The next thing to try is to dilute a new batch of sperm and check to be sure it is active. If neither of these things seem to identify the problem, chances are that the eggs are immature. This can sometimes be diagnosed by seeing a very large nucleus, since an egg that has not yet undergone meiosis will still have a diploid number of chromosomes and a large nucleus.
5. If the eggs are getting fertilized but do not divide, or they divide abnormally, the cause is probably **polyspermy** (fertilization of each egg by more than one sperm). Dilute the sperm, make sure it's still active, and start over.
6. It may be interesting to your students to discuss how different the tempo of sea urchin development is from that of human development. With sea urchins the first division of a fertilized egg occurs in about an hour, and then subsequent divisions occur about every half hour. With humans, however, the first division does not occur until more than a day after fertilization, and at the end of two days, while the sea urchin is already swimming around as a pluteus larva and feeding itself, the human embryo is just getting around to dividing the second time. Moreover, by the time the sea urchin has become a mature adult and is having "babies" of its own (about 18 months), the human infant is nine months old, still wholly dependent on its parents for food and everything else required for survival. The magnitude of these differences in timing makes it seem all the more astonishing how similar in quality many of the chemical changes are that occur in the two kinds of eggs following fertilization.
7. Keeping sea urchins for a prolonged period of time in an inland location is difficult. Even teachers who maintain a salt-water aquarium have often found that adding the

left-over urchins to such an aquarium is a mistake; they can foul the aquarium and cause severe difficulties. The most humane way of killing urchins after you are through using them is to put them in a freezer. After they are dead, you may preserve the tests (shells) by washing them in diluted bleach and setting them out to dry. Students who are squeamish about holding a live urchin may be willing to hold and examine a lifeless test.

REFERENCES

Dr. David Epel of Stanford University has prepared an excellent resource regarding sea urchin development. It can be found at http://www.stanford.edu/group/Urchin/fert.htm. This site takes students through gamete collection, fertilization, and development. It has good animated sequences, lots of useful information, and great ideas for further studies.

Womb with a View (1995), Kendall/Hunt Publishing Company. This reference book for teachers contains several activities related to meiosis and mitosis and is an excellent resource for sea urchin background information. It discusses the historical uses of the sea urchin as well as its anatomy and taxonomy. The book has more detailed ordering information and includes several activities related to meiosis, mitosis, sea urchin fertilization, and sea urchin behavior.

From Egg to Adult: A Report From the Howard Hughes Medical Institute (1992). Available by writing Howard Hughes Medical Institute, 4000 Jones Bridge Road, Chevy Chase, MD 20815-6789. This colorful brochure illustrates beautifully the way in which fruit flies have served as a model system to identify the genes that regulate formation of the human body from head to toe.

ANSWERS TO POSTLAB QUESTIONS STUDENT PAGE 86

1. If an adult sea urchin of one particular species has 14 chromosomes, how many chromosomes would an egg or sperm of that species have?
   It would have seven chromosomes.

2. What do you think would happen if one of the gametes (either the egg or the sperm) had the wrong number of chromosomes? Why?
   The embryo derived from the fertilized egg would not develop normally. This is because an embryo needs to receive an equal number of chromosomes from each parent in order to develop normally.

3. What are some differences between a fertilized and unfertilized egg?
   A fertilized egg has twice as many chromosomes as an unfertilized egg. It also has a fertilization membrane that protects it from being penetrated by a second sperm. It also is capable of dividing and forming a sea urchin larva, something an unfertilized egg cannot do.

4. What is the function of the fertilization membrane? Why would that be important?
It is to prevent a second sperm from fusing with the egg. That is important because an egg that fuses with more than one sperm never develops normally.

5. What happens to the fertilized egg about an hour after fertilization?
It undergoes its first division.

6. Whenever a cell of an embryo divides, how are each of the newly formed cells similar to one another and to the original fertilized egg but different from the unfertilized egg?
They are diploid, whereas the unfertilized egg was haploid.

7. Mitosis and meiosis are essential aspects of the cycle of life and development. Complete the adjacent diagram by writing mitosis or meiosis on the correct lines.
a. meiosis
b-e. mitosis

B.3

The Miracle of Life STUDENT PAGES 87-88

LESSON OVERVIEW

If all went well, the preceding exercise provided your students a chance to watch gamete release, fertilization, and the early development of a sea urchin. Now, thanks to the spectacular cinematography included in this video, which was first shown on *Nova*, they will get to see the equivalent processes taking place in human beings.

TIMELINE

Viewing this video and answering the questions will take an entire 50 minute period.

MATERIALS

The Miracle of Life can be ordered from:
Carolina Biological Supply Company
(800) 334-5551
www.carolina.com
The Miracle of Life, catalog no. BA-49-3555V

ANSWERS TO *THE MIRACLE OF LIFE* QUESTIONS STUDENT PAGES 87-88

1. Describe the journey of the egg as it becomes mature and travels toward the sperm.
   The egg matures in a follicle in the ovary, is released when the follicle ruptures, and is then swept into the fallopian tube, where it is pushed in the direction of the uterus by cilia on the surface of the cells lining the fallopian tubes.

2. Describe the journey of the sperm as they leave their site of origin and travel toward the exterior.
   Sperm are formed in the seminiferous tubules in the testes and stored in the epididymis. During ejaculation they travel through the vas deferens, are mixed with secretions released by the seminal vesicles and prostate gland, and then travel through the urethra and are expelled into the vagina.

3. About how many sperm does a man produce in his lifetime?
   A typical male produces about 400 billion sperm in his lifetime.

4. About how many sperm are released in a single ejaculation?
   About 200-300 million sperm are released in a typical ejaculation.

5. After sperm are released into the vagina, how long are they viable?
   Sperm remain viable in the female reproductive tract for 24-48 hours.

6. Describe the barriers that the sperm face as they travel up the female reproductive tract toward the egg.
   The acidic condition of the vagina kills many sperm, as do protective cells that attack them. Then once they reach the fallopian tubes, the sperm must swim upstream against the current generated by the cilia.

7. Where is the egg when the sperm reach it?
   It is in one of the fallopian tubes.

8. About how many sperm reach the egg?
   Only about 50 sperm ever make it to the vicinity of the egg, and only one enters it.

9. What happens to the sperm after it enters the egg?
   The sperm loses its tail and midpiece, and its head swells and then ruptures, releasing its genetic material into the egg.

10. When does the fertilized egg begin dividing?
    The egg divides for the first time about a day after fertilization.

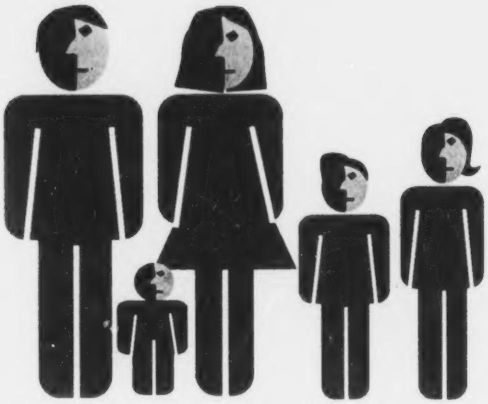
11. What is the fertilized egg called after it divides?
    When the egg is fertilized, it becomes a zygote; then when it begins to divide, it becomes an embryo.

12. How long after fertilization does the embryo implant itself in the uterine wall?
    Implantation in the uterine wall begins about 10 days after fertilization.

13. Describe the human embryo at the following stages:
    4 weeks: It has arm buds and the beginnings of eyes.
    5 weeks: *It has a nose and leg buds.*
    6 weeks: *It is about 1/2 inch long.*
    7 weeks: *It is about 3/4 inch long and has fingers*
    8 weeks: *Bones and joints are visible in its fingers and toes.*
    10 weeks: *It is now about 2 inches long.*
    14 weeks: *It is able to suck its thumb.*
    18 weeks: *Its eyes are still closed, but it can detect light.*

CHAPTER 2

Passing Traits from One Generation to the Next



SECTION C

If All the Kids Have Mom and Dad's Genes, Why Don't They All Look Alike?

Chapter 2: Section C Background

IN SECTION B, YOUR students saw how the fusion of sperm and egg initiates the beautiful and mysterious process by which a genotype gives rise to a phenotype. Now it is time to get the students to contemplate the rules of heredity that relate the phenotype of the offspring to the genotypes of the parents that produced the egg and sperm. Our goal in this section is to help our students understand the answer to the question, If all the kids have Mom and Dad's genes, why don't they all look alike?

The field of inquiry to which your students will be introduced in this section is called Mendelian genetics, because it involves using methods of investigation very similar to the ones Gregor Mendel used more than 150 years ago.

In the first exercise, your students will have the fun of crossing two members of a very charming species of imaginary animals – the Reebops – and producing a young Reebop of distinctive phenotype. Although all the progeny produced by the class will be children of the same pair of parents (Mom and Dad Reebop), it is extremely unlikely that any two of the young Reebops will look exactly alike. In the course of this exercise, your students will have a painless introduction to many of the most important terms and concepts of Mendelian genetics.

Then in the second exercise, they will learn how to use a Punnett square, as well as a very simple mathematical method, to make genetic predictions. In this exercise, they will perform the equivalent of one of the most important tasks a genetic counselor must perform: predicting the probability that two parents of known genotype will have a baby with some particular phenotype.

In the third exercise, genetics will strike much closer to home. The students will pair up to produce an imaginary human baby together, and will discover which of them the baby will resemble with respect to 22 different phenotypic characters.

In the final exercise they will take information from Exercise 3 and use a more complicated Punnett square to make a more complicated genetic prediction.

All in all, this section should not only introduce your students to many important Mendelian-genetic concepts, it should reinforce the concept that genetics (and science in general) can be fun!

C.1

Really Relating to Reebops

STUDENT PAGES 90-94

LESSON OVERVIEW

Reebops (invented by Patti Soderberg at the University of Wisconsin*), are model "organisms" that students can use for a variety of genetic studies. In the initial exercise described here, students receive a set of diploid "chromosomes" that represent the genotypes of a pair of parental Reebops. The students then simulate meiosis, combine the resulting two haploid sets of chromosomes, and then determine phenotypes for individual offspring, which they construct from inexpensive materials. The class then compiles data for this F1 generation and forms hypotheses about the inheritance patterns. This exercise can be carried through a number of generations, if desired, to test various hypotheses.

TIMELINE

Two 50 minute periods.

MATERIALS

For each pair of students provide at least:

- 4 standard white marshmallows
- 1 red miniature marshmallow
- 1 orange miniature marshmallow
- 1 yellow miniature marshmallow
- 3 green miniature marshmallows
- 8 toothpicks
- 2 small nails
- 2 thumbtacks
- 4 red pushpins
- 4 blue pushpins
- 1 short and straight pipecleaner
- 1 long and curled pipecleaner
- 1 set of red and 1 set of green chromosomes in an envelope

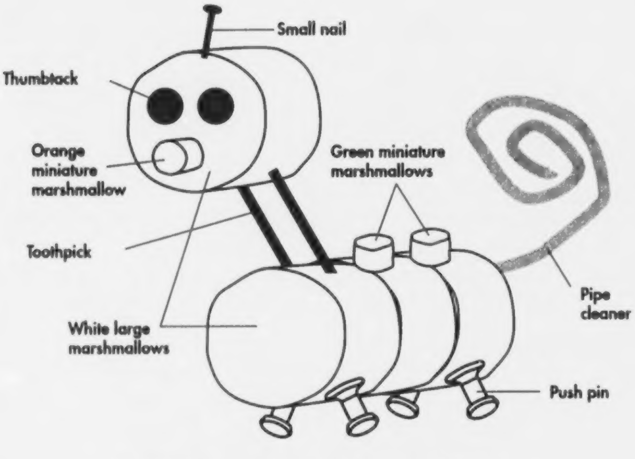
ADVANCE PREPARATION

- Prepare two diploid chromosome sets per pair of students, using the templates provided. For convenience, we have put two sets of chromosomes on one page, but you will need one Mom set on red paper and one Dad set on green paper for each student pair. These chromosomes will be much more durable if they are laminated before they are cut out.
- Assemble Mom and Dad Reebop. The chromosomes indicate that both Mom and Dad Reebop are heterozygous at each locus (or gene pair). So the phenotype of each of them should be as follows: three body segments, one antenna, orange nose, two eyes, two green humps, curly tail, and blue legs (see drawing).

**The lesson included here has been adapted from Patti Soderberg's version with her permission.*

PROCEDURE

Day 1



Toothpicks function as the unseen ligaments and tendons that hold the Reebop together.

- Place body parts in boxes at the front of the room.
- Give each pair of students an envelope with their chromosomes and introduce the class to Mom and Dad Reebop, which you should leave in a conspicuous place during the rest of the exercise. Make sure that the students sort the parental chromosomes in the kit and perform "meiosis" with code letters face down, gathering one haploid set of chromosomes from each parent at random. (They may then put the extra chromosomes back in the envelope without looking at them.) Now they "recombine" the haploid chromosome sets produced by Mom and Dad Reebop to generate the genotype of baby Reebop. After turning their baby's chromosomes letter-side up, they should record this genotype on the worksheet. Then they should use the Genotype-Phenotype Conversion Table to determine (and record) what the baby's phenotype will be. Finally, they should assemble a baby with this phenotype.
- If there is enough time left after the babies have been "born," you may wish to have the students work on the Reebop Review. Alternatively, you may wish to assign it as homework. (Note that it includes an introduction to predicting probabilities with a Punnett square.)

Day 2

- This session is used to compile and discuss the data from the population of Reebop babies in the class.
- Try to work the key concepts of Mendelian genetics into the discussion at appropriate places. See the Genetic Glossary on S93.
- Some teachers may have a tendency to downplay the concept of a locus, because students may initially have a bit of trouble understanding it. But it is very difficult to discuss any but the very simplest of genetic concepts without using the concept of a **locus.** So please work on getting students to understand it. A locus is the chromosomal site where alleles affecting one particular heritable trait are located. A clear example may help: The ABO locus is the place on a particular chromosome where the proteins responsible for the A, B and O blood-group antigens are encoded. The A allele at this locus encodes an enzyme that puts one kind of sugar on a red blood cell (RBC), mak-

ing it type A. The B allele encodes a different form of this enzyme, which puts a different kind of sugar on the RBC, making it type B. The O allele at this locus encodes an inactive form of the enzyme. (RBCs in AB heterozygotes have both kinds of sugar on their surface and type AB blood, whereas OO homozygotes have neither sugar on their cells and thus have type O blood.)

See "Suggested topics for additional discussion" in the next exercise.

ANSWERS TO REEBOP REVIEW STUDENT PAGE 95

1. Define the following terms and give an example of each from this activity. (You may refer to the Genetic Glossary.)
   allele: *one of two or more forms of a gene that can exist at a single locus*
   genotype: *the specific combination of alleles that an individual possesses at one or more loci*
   phenotype: *the outward appearance of an individual with respect to one or more traits that is associated with some particular genotype*
   homozygous: *having two identical alleles at a particular locus*
   heterozygous: *having two different alleles at a particular locus*

2. If a Reebop female with a red nose and a male with a yellow nose marry and have children, what genotype and phenotype for nose color will their children have? (You may refer back to the Reebop Genotype-Phenotype Conversion Table.)
   genotype *Nn*
   phenotype *orange nose*

3. If a Reebop female with one antenna and a male with no antennae marry and have children, what genotypes and phenotypes might their children have with respect to number of antennae?
   genotypes *Aa or aa*
   phenotypes *one antenna or no antennae*

4. If a Reebop female with one antenna and a male with one antenna marry and have children, what is the probability that they will have a baby with no antennae? (If you have difficulty answering this question, check out section C.2.)
   The probability is 1 in 4, or 25%.

5. If a Reebop female with two green humps and a male with two green humps marry and have children, what is the probability that their first baby will have two green humps?
   The probability is 1 in 2, or 50%.

6. If a Reebop female with three green humps and a male with three green humps marry and have children, what is the probability that they will have a baby with two green humps?
   The probability is zero.

7. If a Reebop baby has a straight tail, but both of his parents have curly tails, what are genotypes of the two parents?
With respect to this trait, both parents have the genotype Tt. (Because the baby has the genotype tt, it must have inherited a t allele from each parent. Therefore both parents must be heterozygous.)

ANSWERS TO ANALYSIS OF REEBOP FINDINGS STUDENT PAGES 96-97

1. Describe the phenotypes of Mom and Dad Reebop.
three body segments, one antenna, orange nose, two eyes, two green humps, curly tail, and blue legs.

2. Using the information in the Reebop Genotype-Phenotype Conversion Table, list all of the genotypes that would produce the phenotypes exhibited by Mom and Dad.
DD or Dd, Aa, Nn, EE or Ee, Mm, TT or Tt, LL or Ll.

3. How many of the Reebop babies in your class have the same phenotypes as Mom or Dad?
None

4. Do any two babies in your class have exactly the same phenotypes?
(Probably) no

5. Why do some Reebop babies have traits that are not seen in either Mom or Dad?
These are all traits for which the parents are heterozygous but the babies are homozygous recessive.

6. Which Reebop traits are dominant?
three body segments, two eyes, curly tail, and blue legs

7. Which Reebop traits exhibit codominance?
number of antennae, nose color, and number of humps

8. Use the information you have about the phenotypes of all of the Reebop babies in your class to figure out what the genotypes of Mom and Dad Reebop must be. Write the answer below.
Dd, Aa, Nn, Ee, Mm, Tt, Ll

9. If you know the genotype of the parents, is it possible to predict all of the possible genotypes of babies that they might produce?
yes

10. If you know the genotype of the parents, is it possible to predict the genotype of any particular baby, such as their first one?
Not unless both parents are homozygous at all loci. (If they were both homozygous at all loci, all of their offspring would have the same genotype, but such a situation would be extremely rare in the real world.)

11. The Reebops appear to have only one gene on each chromosome. Do you think this is true of real, living organisms?
Probably not. More than likely they all have many more genes than they have chromosomes.

A a D d E e
L l M m
N n
T t

A a D d E e
L l M m
N n
T t

C.2

Determining Genetic Probabilities with a Punnett Square

STUDENT PAGES 98-99

LESSON OVERVIEW

One of the most important uses of genetic information outside of a classroom or laboratory is to predict the probable genotypes and phenotypes of unborn or potential offspring. For example, if a man and a woman both have siblings who suffer from cystic fibrosis (a heritable disease), they undoubtedly would wonder what their chances would be of having a child with cystic fibrosis if they were to marry and raise a family. We will get into this sort of prediction with respect to human diseases in a later chapter. The Reebops activity provided a great opportunity to introduce the concepts of genetic predictability and probability in a less somber context.

Students saw how many different Reebop phenotypes were generated with only seven allele pairs; from that exercise they should have gained some insight into the enormous phenotypic diversity that can be generated by the twin processes of meiosis and recombination in natural populations of real organisms. It is such phenotypic diversity that serves as the raw material on which natural selection acts to bring about evolutionary change.

The **Punnett square** provides a way for students to visualize all of the genotypic and phenotypic possibilities in a cross between parents of known genotype and to determine the relative probabilities of each of those possibilities. It is a good idea, however, to make the point that a Punnett square is just a way of visually confirming a basic rule of probability that applies to many events, not just sperm and egg fusion. The rule highlighted by the Punnett squares is that **the probability of two events occurring together is equal to the product of their independent probabilities.**

To apply this mathematical rule to the problem being addressed here, one would proceed as follows: The probability that an egg produced by an **Aa** female will contain the **a** allele is 1 in 2, and the probability that a sperm produced by an **Aa** male will contain the **a** allele is 1 in 2; therefore, the probability that a baby produced by this couple will have the **aa** genotype is 1/2 x 1/2, or 1/4.

An advantage of using the Punnett square is that it gives students a way to visualize all of the possible outcomes as sperm and eggs combine in a particular genetic cross. But the mathematical approach of multiplying individual probabilities is applicable to many more situations in science and life.

This lesson will use Punnett squares and mathematical calculations in a guided practice activity. You could easily pick other Reebop traits and have the students work through the same process on their own.

TIMELINE

This exercise will only take about 5 to 10 minutes of class time. But you could assign it as homework, together with a few other problems in Reebop heredity for the students to work out using Punnet squares and/or product-of-probability methods.

C.3

Exploring Human Traits: Create-a-Baby

STUDENT PAGES 100-105

LESSON OVERVIEW

Now that your students have encountered the basic concepts of Mendelian genetics by working with the Reebops, they get to apply them to beings with which they should have even less trouble identifying – their own imaginary offspring! Working in pairs, the students will analyze their own phenotypes and genotypes with respect to 22 visible features of the head and face. These will include dominant/recessive traits, codominant traits, and simple multigenic traits. Having established their own genotypes, they will use a coin toss to determine which alleles they will pass on to their offspring at each locus where they are heterozygous. When alleles from two partners have been recombined in the imaginary baby, they will work out the complete phenotype of the baby and name it. Then each partner will draw a picture of what they think the baby would look like.

TIMELINE

The reading, the phenotype and genotype diagnoses, and the Create-a-Baby Table require 50 minutes to complete. If you chose to have the students do their drawings in class, a second period will be required. However, the drawings and the Create-A-Baby Review could be assigned as homework.

MATERIALS

a coin for each student
extra paper
colored pencils

HINTS AND TROUBLESHOOTING

Many teachers compile all the baby pictures produced that year into a baby book. Students often seem to take more pride in their work when they know it is going to be displayed to the following year's students.

ANSWERS TO CREATE-A-BABY REVIEW STUDENT PAGES 106-107

1. Define each of the following terms:
   chromosome, codominant, diploid, haploid, meiosis, multigenic, recombination

   chromosome: a structure in the nucleus of a eukaryotic cell that contains a linear array of many genes. A chromosome is composed of a single DNA double helix molecule wound around many protein molecules that stabilize it and regulate its function.

   codominant: refers to a pair of alleles, both of which exert an effect on the phenotype when they are present together. In codominance, the heterozygote has a phenotype different from that of either homozygote and sometimes (but not always) is intermediate in phenotype.

   diploid: having two complete sets of chromosomes, one set derived from the mother and one from the father.

   haploid: having only one set of chromosomes (as in a sperm or egg nucleus).

   meiosis: the "reduction division" in which a diploid cell divides to produce haploid cells that will function as gametes (eggs or sperm).

   recombination: the process in which two haploid sets of chromosomes are brought together in a pair of gametes to produce a new diploid offspring. Usually this new diploid will be different in genotype from both of its parents.

2. What was the probability that you and your partner would produce a boy? A girl? Explain.
   There was a 50% probability it would be a boy and a 50% probability it would be a girl, because the gender was determined by the toss of a coin, and the coin had a 50-50 chance of coming down heads versus tails.

3. Explain how it is possible for your baby to have a visible trait that neither you nor your partner have.
   If both partners are heterozygous at a particular locus, the baby could inherit one recessive allele from each parent and exhibit the recessive version of that trait.

4. If you and your partner repeated this exercise and produced another imaginary baby, do you think it would look just the same as the one you produced already? Explain.
   It would probably look quite similar, but not identical (as siblings usually do). This is because in every case where a coin toss was used to decide which allele the baby would get, there is a 50-50 chance that the outcome would be different the second time.

5. A woman who is heterozygous for the chin-dimple trait marries a man without a chin dimple. What are the possible genotypes and phenotypes of their children?
The woman could contribute either a P or a p allele, but the man could contribute only the p allele. So the children could be either Pp (dimpled chin) or pp (no dimple).

6. What is the probability that the man and woman discussed in the preceding question will have a baby with a chin dimple?
50%

7. A man and a woman who are both heterozygous for two traits, the cheek-dimple and the chin-dimple traits, get married. What is the probability that they will have a baby that has cheek dimples but not a chin dimple? (If you have trouble answering this question, check out section C.4.)
The probability that one of their babies will have cheek dimples but not chin dimples is 3 in 16, or 3/16. (One way of obtaining this answer is by performing a 4-by-4 Punnett square. A second way is by multiplying the probability that one of their children will have cheek dimples [3/4] by the probability that one of their children will not have a chin dimple [1/4]; 3/4 x 1/4 = 3/16.)

8. What is the probability that a man with dark blonde hair and a woman with red hair will have a baby with brown hair?
100%! (According to the information used in this exercise, the genotype corresponding to dark blonde hair is rr SS and the genotype corresponding to red hair is RR ss. All of the children of parents with these genotypes will be double heterozygotes: Rr Ss. The phenotype associated with the doubly heterozygous genotype is brown hair.)

C.4

Using a More Complicated Punnett Square STUDENT PAGE 108

LESSON OVERVIEW

Question 7 on the previous lesson leads students into the use of a 4 x 4 Punnett square to deal with alleles at two loci. Once again, after the students have used the Punnett square to answer a question, you should discuss how the answer could also be obtained by multiplying independent probabilities.

For example, the question asked in the Punnett square discussion read, "When a man with an oval face and wavy hair marries a woman with an oval face and wavy hair, what is the probability that they will have a baby with a square face and curly hair?"

The parents both have oval face and wavy hair, so they are both heterozygous at both loci of interest: they both have the genotype **Aa Hh**. Rephrasing the question, what we want to know is, What is the probability that two parents with the genotype **Aa Hh** will have a baby with the genotype **aa HH** (the genotype for square face and curly hair)?

- We can break this problem into two parts. First we ask, What is the probability that the baby will be homozygous recessive **(aa)** at the **"a"** locus? There are two ways we can answer this part of the question.
- We may simply remember that the probability of any two heterozygotes producing a homozygous-recessive offspring is always 1/4. Or we may get the same answer by the process of multiplying the independent probabilities. The probability that the baby will inherit the **a** allele from the mother is 1/2, and the probability that it will inherit the **a** allele from the father is 1/2; therefore, the probability that it will inherit two **a** alleles is 1/2 x 1/2 = 1/4.
- Next we ask, What is the probability that the baby will be homozygous dominant **(HH)** at the **"h"** locus? By one of the two processes that we used above, we establish that the probability that the baby will inherit two dominant alleles at the **h** locus is also 1/4.
- So now, to determine the probability that the baby will have the **aa HH** genotype, we simply multiply the probability that it will be **aa** (1/4) by the probability that it will be **HH** (1/4). 1/4 x 1/4 = 1/16.

In the C.4 exercise, students were guided very carefully through the use of the 4 x 4 Punnett square and given the answers. It would be a good idea to choose a pair of different Create-a-Baby traits and have students perform a Punnett square analysis of those traits (perhaps as a homework assignment).

An advantage of the Punnett square method is that once the diagram has been filled in, one has information regarding the probabilities for all possible genotypes and phenotypes that could result from that particular cross. On the other hand, the advantage of the "product-of-independent-probabilities" method is that it can be applied to many situations in which the Punnett square is not useful, such as many non-genetic probability problems and genetic problems that are much too complex to be solved with a Punnett square.

For example, suppose we wanted to know what the probability is that one of the babies produced by Mom and Dad Reebop would have the homozygous-recessive genotype at all seven loci at which Mom and Dad are heterozygous. Because there are seven loci, we would need a large square containing 2^7, or 128, squares on each side if we were to use the Punnett square method. Completing and analyzing such a huge Punnett square without any errors would be tedious, to say the least. On the other hand, to solve the same question by the product-of-independent-probabilities method is quite simple. We know (or can quickly calculate) that if both parents are heterozygous at any one locus, the probability of the baby being homozygous recessive at that locus is 1/4. Therefore, to calculate what the probability is that the baby will be homozygous recessive at all seven loci at which the Mom and Dad Reebop are heterozygous, all we have to do is calculate the value of $(1/4)^7$. This reveals that the probability of a Reebop baby being homozygous recessive at all seven loci is 1 in 16,384, or 0.006%.

TIMELINE

It will take an average student about 10-15 minutes to read through this exercise carefully enough to really understand it.

SUGGESTED TOPICS FOR ADDITIONAL DISCUSSION

1. Why don't all the babies in either the Reebop or the Create-a-Baby exercise look just like their parents?
   They have different combinations of alleles than their parents have.

2. Does it make any difference whether you get a particular allele from your father or from your mother?
   In nearly all cases it does not. In fact, until a few years ago the answer to this question was thought to be a simple "no." But in recent years it has been discovered that for a few genes (particularly ones that control aspects of very early embryonic development) it does make a difference whether a particular allele was inherited from the father or the mother. However, this is an exception to the general rule, which is that any particular allele has the same effect in the offspring whether it came from the mother or the father.

3. Are dominant traits always more common than recessive traits?
   No. For example, in humans the allele for six fingers and six toes is dominant over the allele for five fingers and five toes, but the **polydactyly** *(extra digits) allele is quite rare.*

4. If you know the genotype of the parents, can you predict the possible genotypes of the babies?
Yes. But when the parents are heterozygous for several different genes (as Mom and Dad Reebop were in the previous exercise, for example), it becomes complicated. When Mom and Dad are both heterozygous at only one locus (Aa), three different genotypes of babies are possible: AA, Aa, or aa. If they are heterozygous at two loci (Aa and Bb), there would are nine possibilities: AABB, AABb, AAbb, AaBB, AaBb, Aabb, aaBB, aaBb, and aabb. So we can quickly see that the relationship is…what? (Answer: There are 3^n *different genotypes possible, where n is the number of loci at which Mom and Dad are heterozygous.) So, since Mom and Dad Reebop were heterozygous at seven loci, how many different genotypes of babies could they have produced? (Answer:* 3^7*, or 2,187! That is why there will very rarely be two Reebop babies of identical genotype produced by a class.)*

If two members of this class had been heterozygous with respect to 20 of the traits that were included in the Create-a-Baby Table, there would have been 3^{20}*, or **more than three billion**, possible genotypes for the offspring they produced. Most humans are probably heterozygous at thousands of different loci, so the number of possible genotypes of their offspring is astronomical. That is why siblings never look exactly alike unless they are identical twins (derived from a single fertilized egg).*

5. Why are there so many different skin and hair colors in humans?
Skin and hair color, like many other human traits, are determined by the effects of more than one gene, so there are many possible combinations. For example, it is thought that alleles at seven different loci have major effects on skin color in humans. So, as we just calculated, if there are only two different alleles at each of these seven loci, there are 2,187 possible genotypes. So at least 2,187 different skin color genotypes are possible in humans. This is without even making any allowance for the effect of sunlight on skin color.

6. Are there only two possible alleles at each locus?
No, there can be any number. As a simple example, there are three common alleles at the ABO blood-group locus. Alleles A and B encode different forms of an enzyme, thereby causing two different kinds of sugars to be put on the surface of red blood cells (RBCs). Alleles A and B are codominant, so a person who is heterozygous for these two alleles has both types of sugar on the RBCs and thus has type AB blood. The O allele (which encodes an inactive form of the enzyme and thus is unable to put any sugar on the surface of the RBCs) is recessive to both the A and B alleles.
As a more complex example, in humans there are more than 100 alleles ~~at most~~ of the 11 histocompatability loci, which encode proteins on the surfaces of cells in most human tissues. It is differences in these proteins between donor and recipient cells that cause the immune system to recognize grafted tissues as foreign and attack them. With 100 alleles at 11 loci, there would be 100^{11}*, or* 10^{22}*, possible combinations of different histocompatability alleles, which is far greater than the number of human beings that have ever been born. That is why it is impossible (except in the case of identical twins) to find an organ donor who is a perfect match for someone who needs an organ graft.*

7. Mom and Dad Reebop have only one gene on each chromosome. How many different genes do humans have on a chromosome?
   As a result of the sequencing of the human genome, which was completed in 2001, we now know that humans have about 35,000 genes per haploid chromosome set. These are distributed on 23 kinds of chromosomes that differ quite a bit in size, suggesting that the number of genes per chromosome is probably also quite variable. So a good guess would be that on average a human chromosome probably contains around 1,500 genes, but that the range might be from about 750 to about 3,000.

HINTS AND TROUBLESHOOTING

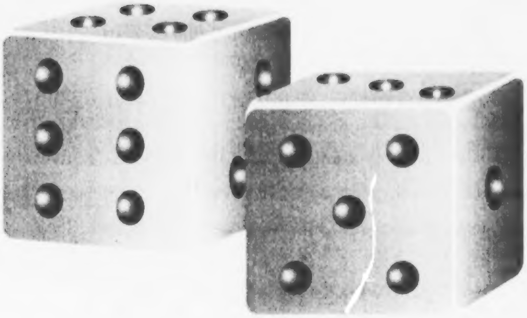
Create-a-Baby is another fun exercise and a good complement to the Reebops. It reinforces students' newly gained knowledge of genetic concepts and terminology by referring to organisms with which they have no trouble identifying: themselves, their friends, and their future progeny.

You can contribute to the mutually reinforcing nature of these two exercises by tying the two discussions together as much as possible. Among the major take-home messages should be the following: (a) as a result of meiosis and recombination, a pair of sexually reproducing organisms have the potential to produce progeny with many different genotypes and phenotypes, but (b) if we know the genotype of the parents, we can predict how probable it is that one of their offspring will have any particular genotype of interest.

This would be a good time to provide your students with copies of Appendix C ("Some Presumably Simple Heritable Human Traits"). If you do, be sure to call their attention to the introduction, which indicates some of the reasons that the information in this table should be considered tentative.

CHAPTER 2

Passing Traits from One Generation to the Next



SECTION D

How Are Genetic Experiments Actually Performed?

Chapter 2: Section D Background

IMAGINARY ORGANISMS ARE FUN. They can also be instructive, as we saw in Section C. But Mendelian genetics is not about imaginary organisms any more than chemistry is about imaginary elements. So it's time to introduce your students to some real Mendelian-genetic experiments, with some real, live organisms.

Although all sexually reproducing eukaryotic organisms that have been studied adhere to the principles of Mendelian genetics, there are a very limited number of them that are suitable for performing Mendelian genetic experiments in a secondary-school classroom. In order to be useful in such a context, organisms must be quite small (so a large number of them can be produced and maintained in a small space). They must be relatively easy to raise in captivity (so teachers and students with little prior experience can work with them effectively). They must have a relatively rapid sexual reproductive cycle (so progeny from a sexual cross can be produced and analyzed within the confines of a standard school term). And they must be available in strains exhibiting reproducible visible differences that students can readily distinguish (so students can easily collect the data that will enable them to deduce the genetic basis for the phenotypic traits(s) in question).

Fruit flies meet all of the above criteria and therefore probably have been used for classroom Mendelian genetic experiments more than all other organisms combined. Nevertheless, they do not always meet one other criterion not listed above: to be actually adopted for classroom genetic studies, organisms must be aesthetically acceptable to the teacher. Our experience indicates that many teachers say they prefer not to have rogue fruit flies buzzing around their classrooms all semester or refuse to use fruit flies for some other reason. So the exercises developed here are based on two very different kinds of organisms that meet the above criteria: a fungus (baker's yeast) and a plant that was developed for studying genetics in the classroom (Wisconsin Fast Plants).

As you will read in the Overview to Section D.1, baker's yeast has become one of the most intensively studied organisms in the world. Here your students will use it to generate a Punnett square that illustrates the results of a monohybrid cross, not with letters on paper, but with live organisms that are growing and exhibiting two readily distinguishable diploid phenotypes.

As you should already have read by now (on the second page of this manual), *fast* is a relative term when it comes to plants. If you have made the recommended preparations, we believe your students will have a truly rewarding experience, as they see the results of a dihybrid cross unfold before their eyes. However, we do provide an alternative, less time-consuming option that will permit your students to gain some of the benefits of the Fast Plants, even if you are unable to devote as much time to this topic as the two-generation Fast Plant exercise requires.

D.1

A Colorful Experiment in Yeast Genetics

STUDENT PAGES 112-119

LESSON OVERVIEW

In this exercise, students perform a genetic cross that provides information about a single gene trait. Haploid strains of baker's yeast (*Saccharomyces cerevisiae*) come in two mating types, A and α (alpha), each of which is available in red and white varieties. Therefore, students can use yeast matings to produce a visible Punnett square.

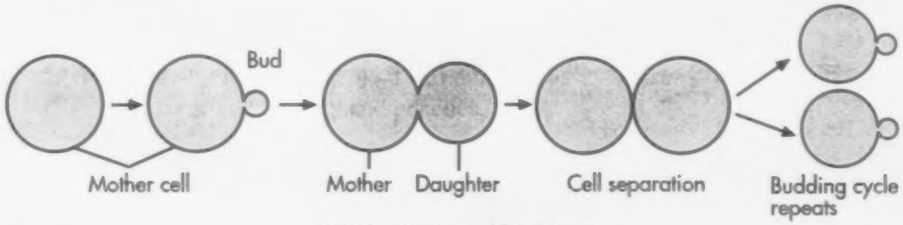
BACKGROUND

Baker's yeast is a unicellular organism that can be grown on culture plates like bacteria. Nevertheless, it is a genuine eukaryotic organism with a nucleus, mitochondria, and many other features shared by all eukaryotic cells. This, combined with its interesting life cycle, which involves an alternation of haploid and diploid phases, makes it a good model organism for studying basic cell biology and genetics.

Yeast was the first eukaryotic organism to have its DNA completely sequenced as part of the Human Genome Project. The reason that yeast was included in the Human Genome Project is because it known to have thousands of genes that are extremely similar to those of humans, but it is much easier to study the function of many of those genes and their products in yeast cells than it is in human beings. Yeast is the most intensively studied eukaryotic organism. Nevertheless, thousands of yeast genes of unknown function were discovered as a result of the DNA sequencing. Now that the sequence of each of those genes is known, work has begun to understand all of their functions.

Cell Growth and Division

Baker's yeast is known as a **budding yeast.** This refers to its unusual form of cell division. It does not just grow larger and then divide in the middle, as many animal and plant cells do. Instead, it forms a small bud at some point on the surface of each **mother cell.** This bud, or **daughter cell,** grows until it is as large as the mother cell, and then it pinches off



Cell Division in Budding Yeast

as a separate cell. (By then, the chromosomes will have been replicated, the nucleus will have undergone mitosis, and the bud will have received a nucleus identical to that in the mother cell.) Under good growth conditions, both the mother cell and the daughter cell (now about to become a mother cell itself) begin to bud again as soon as they have separated. Both haploid and diploid cells of baker's yeast divide this way.

Sexual Reproduction and Beginning of the Diploid Phase

Saccharomyces cerevisiae has a well-characterized sexual cycle, which is illustrated on S113. A haploid cell population consists of cells of one of two mating types, usually referred to as **a** (little a) and **α** (alpha). But because of the difficulty students may have in distinguishing **a** and **α** in writing, we will refer to the two mating types as **A** (capital *a*) and **α** (alpha) throughout the rest of this exercise. Although haploid cells can reproduce asexually (by budding) indefinitely, they also can act as gametes and fuse with cells of opposite mating type to form a diploid.

Each mating type releases a chemical substance that prepares cells of the other mating type for sexual fusion. Thus, as soon as cells of opposite mating type encounter one another, they initiate a series of cellular events that will lead to **conjugation** in which an **A** cell fuses with an **α** cell.

Sporulation and Beginning of the Haploid Phase

Given suitable conditions and adequate nutrients, diploid yeast cells can grow and divide mitotically for an indefinite period. However, if a diploid culture becomes nutritionally deprived, growth and mitotic division ceases, and the cells prepare to undergo meiosis and **sporulation** or spore formation. As a result of meiosis, four haploid cells are produced. These four haploid cells develop as four **ascospores,** dormant, resistant cells, within the wall of the original diploid cell. Sporulation takes at least 15 hours. If ascospores are simply returned to a nutritionally adequate environment, they will germinate and begin to reproduce asexually. But because both mating types will be present under these conditions, they will quickly mate and reform diploids.

On the other hand, if the ascospores are separated from one another while they are still dormant, each will form a stable haploid culture of a single mating type when it is returned to rich medium. This is how the haploid strains that your students will be using were produced.

The Basis for the Red Phenotype in Yeast

In most organisms, presence of color is a dominant trait and colorless (white or albino) is recessive. This is because the colorless trait is usually caused by a mutant gene that encodes an inactive form of an enzyme required for producing a normal pigment. Even when the enzyme encoded by the mutant allele is totally inactive, the heterozygote usually is normal in color, because the enzyme acts as a catalyst, and therefore half the usual amount of enzyme is enough to make the usual amount of pigment. In some cases that is not true, so the traits exhibit codominance, and the heterozygote is intermediate in color between the two homozygotes. (For example, it has pink flowers instead of red or white.)

Yeast normally are not colored. The red strain that will be used in this exercise is unusual in that regard. It has a mutation of a gene that encodes an enzyme required for one particular step in a series of reactions that normally produces the nitrogenous base adenine. When this mutant gene is present in a haploid strain or in the homozygous condition in a diploid strain, this step in adenine synthesis cannot take place.* As a consequence, the compound that is produced in the preceding reaction accumulates in the cell. And as luck would have it, this accumulating intermediate product causes the cell to turn red. However, in a diploid cell that has one copy of the wild-type gene, enough functional enzyme is produced to prevent accumulation of the intermediate. Thus, the cell remains colorless. Or, putting it differently, white is dominant.

Experience indicates that, by one means or another, most students have developed a sense of the usual dominance relationship between color and colorless alleles and therefore will predict that the heterozygous diploid yeast will be either red or pink. The contrast between what most students usually predict and what they actually observe often provides a good opening for discussing the scientific approach as a way of testing assumptions and hypotheses.

REFERENCE

Handbook for Using Yeast to Teach Genetics, T.R. Manney and M.L. Manney. 1991. Manhattan, KS, Kansas State University Department of Physics.
This handbook and the accompanying video have several interesting experiments for further study.
The Genes We Share with Yeast, Flies, Worms and Mice: New Clues to Human Health and Disease. (2001). This booklet contains an up-to-date discussion of the way in which baker's yeast is being used to obtain important new insights into human genetics. A more complete description and ordering information are given on page T106.

TIMELINE

The terms *Day One, Day Two,* and *Day Three* in this exercise are based on the assumption that you will be able to incubate the culture plates at 30° C. If you do not have a 30° incubator, incubate the dishes at room temperature, but allow the yeast to grow for two days between steps, instead of one.

Day One About 15 minutes. Students label their Petri dishes and transfer haploid yeast of four types from stock plates to their own test plates.

Day Two 15-30 minutes. After the haploid cultures have grown enough on the Petri dishes to show their color clearly, students will set up their crosses and then complete the Day Two Worksheet.

Day Three About 15 minutes. Students examine the results of their crosses, record observations and complete the Day Three Worksheet.
(If most of the lab groups find that their cultures have not grown enough to make the color of each diploid entirely clear, all groups should allow their cultures to grow another day before continuing with the analysis.)

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*Adenine is essential for life. It is required for making DNA, RNA, ATP and several other very important cellular components. Thus, the red yeast strain could not grow if you did not provide adenine. However, the yeast extract that you included in the medium provides all of the adenine that the mutant strain needs for normal growth.

MATERIALS

For each group of four students:

- 1 petri dish containing yeast growth medium (YEAD)
- marking pen
- a packet of sterile toothpicks
- culture of Red, Mating type **A** yeast
- culture of Red, Mating type α yeast
- culture of White, Mating type **A** yeast
- culture of White, Mating type α yeast
- waste container
- disinfectant

The four strains of yeast can be ordered from:
Carolina Biological
(800) 334-5551
www.carolina.com

Mating type	Color	Strain Designation	Catalog number
A	red (R)	HA2	BA-17-3624
A	white (W)	HAT	BA-17-3630
α	red (R)	HB2	BA-17-3626
α	white (W)	HBT	BA-17-3631

At the same time that you order the above yeast strains, order the premixed powder that you will use to prepare the medium on which the yeast will be grown: Carolina cat. no. BA-17-3653 Yeast-Extract Adenine Dextrose (enough to make 2 liters of YEAD medium). You should also order 10 ml of 10 mg/ml Gentamycin solution (Cat. # G1272) from Sigma, P.O. Box 14508, St. Louis, MO 63178, (800) 325-3010, www.sigma-aldrich.com.

ADVANCE PREPARATION

NOTE CAREFULLY: Preparations for this exercise will not occupy a lot of your time, but at several points culture plates will need to sit for several days to a week at a time. Therefore, you need to begin preparations for this experiment at least two weeks in advance, and if you do not have an incubator in which you can incubate the plates at 30°C, you should start preparations at least three weeks in advance. If plates and cultures are ready before they are needed, they will keep. But if they are not ready when you want to use them, there will be nothing you can do to rush them along!

1. Preparation of yeast extract/adenine/dextrose plates (YEAD plates) for culturing the yeast. You will need four YEAD plates for your own use, plus five times as many plates as there will be student groups in your largest class, plus one plate for each group of students in each of your other classes. For example, assuming that you have seven groups of students in your largest class and six groups of students in each of your other three classes, you will need to prepare 4 + (5 x 7) + (1 x 3 x 6) = 57 YEAD plates. But because YEAD plates are very easily contaminated, we recommend making a few extras. The general procedure for pouring YEAD plates is similar to the one that you used to prepare nutrient agar (NA) plates for *E. coli* (Chapter 1, Section E). The major difference is that because YEAD is richer in nutrients than NA medium is, more of the bacteria and fungi that are present in room air can grow on YEAD plates than

can grow on NA plates. The gentamycin that we recommend adding will greatly reduce the chance of bacterial contamination, but the plates will nevertheless be likely to become contaminated with airborne fungal spores unless they are poured and used with great care.

It is possible to buy the individual ingredients for YEAD and weigh them all out for each batch of medium. But because you will probably need to make several batches of medium, that could become tedious. We strongly recommend that you buy premixed YEAD powder from Carolina Biological Supply Company as noted earlier.

Below are the materials and steps required to make a batch of 12 dishes:

- 15 g of YEAD powder
- 300 ml deionized water
- 500 ml flask
- piece of aluminum foil
- autoclave or pressure cooker
- hot water
- Gentamycin solution (10 mg/ml)
- 1000 µl micropipettor with sterile tip
- 12 petri dishes
- disinfectant
- 1 Bunsen burner or alcohol burner

a. Weigh out 15 g of YEAD powder.
b. Add 300 ml of deionized water to a 500 ml flask. Swirl the flask while adding the powder slowly.
c. Cover the flask with aluminum foil. Sterilize 30 min at 15 psi in an autoclave or pressure cooker
d. While medium is being sterilized, wipe down a flat surface with disinfectant and spread out 12 petri dishes on it.
e. Set up a Bunsen burner or alcohol burner near the petri dishes.
f. Allow flask containing sterile YEAD to cool just enough that you can handle it comfortably. Use a micropipettor with a sterile tip to add 450 µl of gentamycin solution (10 mg/ml) to the medium. Swirl the flask to ensure that the contents are thoroughly mixed.
g. Light the Bunsen or alcohol burner. Remove the foil lid from the flask. Pass the mouth of the flask through the flame.
h. Lift the lid of a petri dish, fill it about half way with YEAD medium, and cover it back up quickly.
i. Repeat the preceding two steps until all twelve plates have been poured. Fill the flask with hot water to simplify cleaning later.

Repeat the above procedure as many times as necessary, until you have enough plates for all of your classes, plus a few to spare. If necessary, carefully stack one set of plates four-high and move them aside, in order to make space for pouring the next set of 12 plates.

After all plates have solidified, they should be spread out in a place where they can be left undisturbed for a day or two to dry out. (YEAD plates have a tendency to accumulate a great deal of moisture on their lids initially. If this moisture is not allowed to evaporate before the plates are stored or used, contamination with unwanted organisms is almost inevitable.) When all, or nearly all, of the condensation has disappeared from their lids, the YEAD plates can be turned upside down, wrapped (either in the plastic sleeves from which the petri dishes came or in plastic wrap) to prevent further drying. They can then be stored upside down in the refrigerator until they are needed.

2. Preparation of stock culture plates. The yeast cultures from Carolina Biological will arrive in small vials. Use them to establish petri dish subcultures, using techniques similar to those you used to subculture *E. coli* for "Shine On!" (Chapter 1, Section E.2.c). The difference here is that you will grow your yeast subcultures on the YEAD plates that you have prepared in step 1. You will greatly decrease the chances that you will contaminate your stock subcultures (and thereby possibly blow the whole exercise!) if you keep your YEAD plates upside down while streaking the yeast out on the surface of the agar. This will seem a bit difficult to do at first. However, it is the technique that you will be asking your students to use later. So some practice now might come in handy later.

   Be sure to label and date the bottom of each subculture plate as you prepare it. Rather than using the catalog numbers or the strain codes that are used by Carolina Biological to identify the cultures, label your plates with the clear, simple designations that you will have your students use, as shown in the following table:

Catalog number	Carolina strain code	Your label
BA-17-3624	HA2	Red, Mating type A
BA-17-3630	HAT	White, Mating type A
BA-17-3626	HB2	Red, Mating type α
BA-17-3631	HBT	White, Mating type α

   If you have a thermostatically adjustable incubator, set it for 30°C for growing yeast cultures. Otherwise, grow them in whatever place you can find that is closest to 30°C. At 30°C, it should take only two to three days for the white yeast colonies to grow to the extent that they have a rich, creamy consistency and an ivory-white color. At this point they will be ready to use in the next step. The red colonies, however, probably will not look as lush and creamy in consistency as the white colonies and will not be ready for use. If that is the case, put the white yeast in the refrigerator and allow the red ones to grow another day or two, until they also have a rich, creamy appearance and are very dark pink or red in color. If you grow the yeast at room temperature, it might take twice as long to get cultures that are ready to use. The cooler the spot where you grow them, the longer it will be before you are ready for the next step. On the other hand, you should not assume that this means that the higher the temperature, the better. Yeast grow better in an incubator set at 30°C than one set at 37°C, for example.

   Once the red and the white strains on your stock plates have all produced rich, creamy-looking colonies, use them to make one subculture of each strain for each student group in your largest class. (All of your classes will be able to work from the same set of stock plates.) Be sure that you label each plate carefully as you set it up. After establishing the second generation of subcultures for your classes, wrap the first generation plates in plastic film and store them in the refrigerator. It will take just about as long for your second generation plates to reach a useable condition as it did your first generation plates. Once again, permit the red strains to grow longer than the white strains if necessary. If these subcultures are ready for use before your class is ready for them, wrap them in plastic film and refrigerate them; they will hold for weeks with no apparent loss of viability. (We do not recommend trying to hold them over from one year to the next, however.)

3. Sterile toothpicks. Provide each lab group with an unopened box of toothpicks that has just had one corner cut off with a razor blade, so that toothpicks can be shaken out one at a time. (Toothpicks in an unopened package are essentially sterile, so they do not require any additional sterilization.) As long as no contaminated toothpicks have been put back in the package, the package can be passed on from class to class with no problem.

4. Disinfectant. Prepare disinfectant spray bottles as for Chapter 1, Section E.2.

HINTS AND TROUBLESHOOTING

1. The YEAD plates get contaminated easily, so always make a few extra and emphasize the importance of working with them carefully to avoid contamination. Experience indicates that they will have much lower contamination levels if students keep the plates upside down while working with them. As mentioned above, you should practice this technique in advance and then demonstrate it to the class.
2. Unless students are cautioned against doing so, they will tend to ignore the instructions and use as much yeast as they can possibly load on the end of a toothpick to set up each cross. Emphasize that "more is better" does not apply to this case. Indeed, if there are too many haploid cells present, they will interfere with the growth of the diploid cells and will obscure the outcome of the cross. Emphasize that each cross should be set up with just enough yeast of each mating type to be barely visible. Then also emphasize the importance of mixing the two kinds of cells together thoroughly with the second toothpick.
3. After students have set up their crosses and completed the Day 2 Worksheet, have them discuss it as a class. Most students will think either that red will be the dominant color, so that the diploids will be red, or that red and white will be codominant, so that the diploids will be pink. Actually white is dominant, as explained above, but don't reveal this to the class yet.
4. If students have a colony that is mostly white, but with a red center, have them incubate the plate another day, by which time the white diploids should overgrow the red haploids, clarifying the result.
5. At the end of the exercise, have the students open their plates, spray them with bleach, reclose them, tape them shut, and dispose of them in a standard trash container.

DAY 2 WORK SHEET STUDENT PAGE 116

After each group of students has predicted the outcomes of the crosses, have them discuss their predictions as a group. Make a tally of the class predictions.

ANSWERS TO DAY 3 WORKSHEET STUDENT PAGE 118

1. Use the results you observed for your yeast crosses to fill in the blanks on the diagram below:

		Mating type A	
		R	W
Mating type α	R	Genotype RR Phenotype Red	Genotype WR Phenotype White
	W	Genotype RW Phenotype White	Genotype WW Phenotype White

2. What ratio of phenotypes did you observe as a result of the four crosses you performed?
1 red to 3 white

3. What does this indicate about which allele is dominant and which is recessive?
White is dominant, and red is recessive.

4. Is this what you predicted on your Day 2 Worksheet?
(You might want to discuss the class tally here and use the disparity between predictions and observations to discuss the strength of the scientific approach as a way to test – and falsify – hypotheses.)

5. In the table below, list what you predicted and what you observed for each of the four crosses.
Did anyone in the class predict the outcome correctly?

6. If your predicted and observed phenotypes do not agree, how can you account for that, and can you propose a good hypothesis to account for the results you actually observed?
See what students come up with, but then tell them what biologists have discovered about the basis for the color of the red strain. (See the Background Information for an explanation of why red is recessive to white.)

7. If you have come up with a new hypothesis, can you think of a way to test it?
It should be interesting to see what your students come up with here.

D.2

Experimenting with Wisconsin Fast Plants

STUDENT PAGES 120-156

LESSON OVERVIEW

Paul Williams, a plant biologist at the University of Wisconsin, performed an exceptional service for biology teachers and students everywhere when he devoted 15 years to developing the Wisconsin Fast Plants. These plants have made it possible for students to carry out meaningful experiments in plant genetics in less than a semester.

Most plants take at least one full growing season — six months to a year — to go through a full life cycle. (That is, to go from a seed to an adult plant with mature seeds.) Furthermore, most plants get so large by the time that they produce mature seeds that you could not grow more than a few of them in a standard classroom. In contrast, Fast Plants go from seed to seed in about six weeks, and they are so small when they are full-grown that hundreds can be kept in just a few square feet.

Another name for Wisconsin Fast Plants is **rapid-cycling brassicas**. *Brassica* is the plant genus that includes such familiar vegetables as broccoli, cabbage, cauliflower, collards, kale, kohlrabi, and mustard greens (in addition to the rape seed plant, from which the increasingly popular canola oil is obtained). The species of *Brassica* from which the Fast Plants were derived is *B. rapa*, a species from which bok choi, Chinese cabbage and turnips also were derived. Professor Williams planted seeds that had been collected from thousands of brassica plants around the globe, and observed their offspring carefully. The plants of each species that flowered and set seed most rapidly were crossed with one another. This selection process, continued for many generations, resulted in *B. rapa* plants that germinated within a day or two, flowered within two weeks, and had mature seed in less than six weeks. These became the Wisconsin Fast Plants used for teaching, but Fast Plants belonging to five other species of *Brassica* were also produced for use in professional plant research.

Seeds of a number of Fast Plant mutants are now commercially available. Among the mutants with homozygous-recessive phenotypes that can be easily recognized are the following: The *rosette* mutant is much shorter than a wild-type plant because its **internodes** (the regions of stem between successive leaves) fail to elongate. The *elongated internode* mutant has the opposite abnormality and thus is much taller than a wild-type plant. The *petite* mutant is about half as tall as a wild-type plant as a result of a reduction in size of nearly all plant parts, not just an internode abnormality. The *anthocyaninless* mutant lacks the purple-red pigment, **anthocyanin**, that is normally present to a varying degree in the stems, leaves, and various other plant parts; thus these plants have a much brighter, clearer green color than wild-type plants do. On the other hand, the *yellow-green* mutant, as its name suggests, has a less intense green color than wild-type plants do.

The Mendelian-genetics experiment that is outlined in this unit involves a **dihybrid cross**: a cross involving alleles at two different loci (in this case, the *anthocyaninless* and the *yellow-green* loci). It will provide your students with an opportunity to rediscover Mendel s law of independent assortment of alleles at two loci.

The term *law* is put in quotation marks because independent assortment of alleles at two loci is not a basic law of heredity as Gregor Mendel believed it was. The phenomenon of independent assortment (which Mendel observed in every dihybrid cross that he analyzed) is only observed when the two loci being studied are located either on separate chromosomes or far apart on a single chromosome. When the two loci being studied are located near one another on a single chromosome they do not exhibit independent assortment; they exhibit linkage, as will be explained in section 2.E.

Symbols Used to Identify Genotypes and Phenotypes of Fast Plants (and Many Other Organisms)

Wherever you look for information about Fast Plants — whether it is in the Carolina Biological catalog, in information distributed by the Wisconsin Fast Plants Project, or even in the ordering information provided in the MATERIALS section of this unit — you will find symbols used to identify the various Fast Plant genes that look a bit more complicated than the symbols that we ve been using to identify genes and their alleles earlier in this chapter (in the Reebop and the Create-a-Baby exercises, for example).

Be assured that these unfamiliar symbols are not being introduced just to complicate your life as a teacher! They are the kind of symbols that geneticists around the world have agreed to use to symbolize the genotypes and phenotypes of organisms involved in genetic studies. After explaining why such symbols are used, and how they are used, we will suggest how you can avoid introducing such apparent complications into your classroom, if you prefer.

When students are introduced to genetics, they and their teachers are usually given a very simple and easily understood set of symbols to use for representing dominant and recessive alleles. This is what we usually call the **big-A-little-a convention**; a single capital letter represents the dominant allele associated with some particular trait, and the corresponding lower case letter represents the corresponding recessive allele. For example, *T* was used for the dominant Reebop curly tail allele, whereas *t* was used for the recessive straight tail allele (see S94). In contrast, the symbols used for the various Fast Plant genes all use three letters.

Why can t geneticists leave well enough alone and stick with the simple, easily understood big-A-little-a convention for naming genes? One reason is that such a convention only provides a way of naming 26 genes per organism, and even the simplest real organisms have thousands of genes. A second reason is that in most cases a single letter conveys no information about the function of the gene for which it stands. For example, who would guess that *D* stands for a gene that determines the number of segments in the body of a Reebop — without looking it up in the Reebop genotype-phenotype table?

In contrast, in the **three-letter convention** used to identify genes of Fast Plants (and many other organisms), the symbols used for genes are usually abbreviations of the phenotypic traits that they influence. As a result, the gene symbols convey useful information. For example, one of the genes your students will study in this exercise controls the synthesis of the purple-red pigment, anthocyanin. In plants that are homozygous for the recessive allele at this locus, no anthocyanin can be made. Therefore, the symbol for this recessive allele is ***anl,*** which is short for *anthocyaninless*. Similarly, the second trait that will be studied gives the leaves of homozygous-recessive plants a yellow-green color, instead of the usual dark green color. So the symbol for the recessive allele in this case is ***ygr,*** which is short for *yellow-green*.

Like the big-A-little-a convention, the three-letter convention distinguishes the dominant and recessive alleles by use of capital and lower case letters, respectively. Thus, the dominant and recessive alleles at the *anthocyaninless* locus are symbolized ***ANL*** and ***anl,*** respectively. And the dominant and recessive alleles at the *yellow-green* locus are ***YGR*** and ***ygr,*** respectively.

Notice that in the three-letter convention the allele symbols, as well as the names of the genes themselves, are always italicized (or underlined when handwritten). This is because there is a special use for the corresponding nonitalicized symbols: to provide a shorthand identification of phenotypes. Here is how it works:

- The recessive (mutant) phenotype is identified by three non-italicized letters, of which only the first one is capitalized. For example, **Anl** stands for the anthocyaninless (no-purple color) phenotype of the homozygous recessive (*anl/anl*) plants.
- The dominant (wild-type) phenotype is identified by the same three-letter symbol, followed by a superscript-plus sign. For example, **Anl+** stands for the anthocyanin-containing (purple stems, etc.) phenotype exhibited by both the homozygous dominant (*ANL/ANL*) and the heterozygous (*ANL/anl*) plants.

The table below may help you visualize all of these relationships:

Trait being considered	Dominant allele	Recessive allele	Dominant phenotype	Recessive phenotype
Presence versus absence of purple anthocyanin	*ANL*	*anl*	(purple stems, etc.) Anl+	(anthocyaninless) Anl
Yellow-green versus dark green	*YGR*	*ygr*	(dark green leaves) Ygr+	(yellow-green leaves) Ygr

You need to understand the three-letter convention to interpret the symbols for genotypes and phenotypes in the Fast Plant section of the Carolina Biological catalog or in literature distributed by the Wisconsin Fast Plant Project. However, you are free to decide whether you will introduce these symbols to your students or whether you will continue to use the big-A-little-a convention in your classroom. If you prefer, you may have your students use A and a (instead of *ANL* and *anl*) for the dominant and recessive alleles at the anthocyaninless locus and B and b (instead of *YGR* and *ygr*) for the dominant and recessive alleles at the yellow-green locus. The choice is yours.

TIMELINE

Some Important Scheduling Considerations

Fast Plants provide a virtually unrivaled opportunity for students to perform a genuine study in Mendelian genetics in a standard classroom setting. However, fast is a relative term when applied to plants. Although Wisconsin Fast Plants have a generation time of less than one-fourth that of most annual plants, it still takes at least seven weeks to complete a two-generation Mendelian-genetics experiment with them. Some teachers decide that they cannot fit an experiment of such length into their curriculum — even though the plants will require little attention during most of the seven weeks. Recognizing this, we provide two options for conducting the Fast Plant experiment. Details of each option will be given later, but here we will briefly outline both options, so that you can consider which of them better suits your schedule and your teaching philosophy.

Outline of Option 1: A Seven-Week, Two-Generation Experiment

1. About seven weeks before you expect to finish the rest of Chapter 2 have your students plant their F_1 Fast Plant seeds. Do not discuss the genetic aspects of the experiment; only tell them that they are starting an exercise in plant growth and development that will take on additional meaning later.
2. Day 2: The seeds have germinated, and the students watch the plants develop.
3. Week 2: The plants are flowering, so the students pollinate them like busy little bees.
4. Week 5: The F_2 seeds are mature, and the plants are dried out.
5. Week 6: The F_2 seeds are dry and the students plant the seeds.
6. Week 7: The F_2 plants germinate, and the students quickly see that (in distinction to the F_1 plants) the F_2 plants are not all alike. While many will appear wild-type (as their F_1 parents did), some will be *anthocyaninless*, others will be *yellow-green*, and yet others will be both *anthocyaninless* and *yellow-green*. They record the number of plants in each of these categories and then analyze the data to see how closely they correspond to the ratios predicted by Mendel s law of independent assortment.

Total elapsed time: about seven weeks.

Outline of Option 2: A One-Week Analysis of the F_2 Generation

1. Students begin with Step 5 of the above outline, when they plant F_2 seeds that you have purchased and provided to them. As controls, they will also plant seeds from the parental and F_1 generations.
2. Within the first week the students see the same set of phenotypes as the students performing Option 1 do, record the same kind of data, and perform the same kind of data analysis.

Total elapsed time: about one week. If you are already teaching from this manual when you read this page, there is little doubt: Option 2 is for you. (But consider Option 1 for next year!)

PROS AND CONS OF OPTION 1

The major advantage of Option 1 is that the students can follow the F_1 plants through an entire life cycle, pollinate the F_1 flowers, watch them produce seeds, and then harvest and plant those seeds to determine what kinds of plants will be produced in the F_2 generation. This will probably be the first opportunity for most of your students to follow a plant through its entire life cycle. In addition, Option 1 will allow your students to observe one of the most fundamental facts of life: namely, that certain heritable abnormalities can be carried in a latent, invisible form by one generation of individuals, only to appear in a very visible — and sometimes devastating — form in their progeny.

The disadvantages of Option 1 are (a) that it must be started about seven weeks before the students obtain genetic data for analysis, and (b) during the intervening weeks the students will need to water and observe their plants from time to time, even though they will be engaged in other kinds of learning activities in your classroom.

PROS AND CONS OF OPTION 2

The advantage of Option 2 is that your students can collect and analyze the very same kind of genetic data as with Option 1, but in one week instead of seven.

The disadvantage of Option 2 is that it is much more of a cookbook experiment than Option 1. They do not have the opportunity of seeing any phase of the plant life cycle other than the first few days of seedling growth. More importantly, they do not have the opportunity of harvesting their own F_2 seeds from the F_1 plants they raised. Instead, they are handed several sets of seeds and told what the relationship among them is.

MATERIALS OPTION 1

Seeds required per 12 student groups (groups of four are recommended):

Catalog number	No. of seeds	Phenotype	Genotype	Genetic role in exp.
BA-15-8812	50	anthocyaninless (Anl, Ygr+)	*anl/anl, YGR/YGR*	P_A
BA-15-8818	50	yellow-green (Anl+, Ygr)	*ANL/ANL, ygr/ygr*	P_B
Two packs of BA-15-8891	2 x 200	wild type (Anl+, Ygr+)	*anl/ANL, YGR/ygr*	F_1
BA-15-8888	250	segregating all four phenotypes	all of the above and below	F_2 (optional backup)
BA-15-8842	50	anthocyaninless, yellow-green (Anl, Ygr)	*anl/anl, ygr/ygr*	double-mutant

Carolina Biological Supply Company 1-800-334-5551 or Fax 1-800-222-7112

For all classes combined:

- 6 liters of potting mix (1 part Peatlite, RediEarth, or JiffyMix to 1 part medium vermiculite)
- Peter s Professional Fertilizer 20-20-20, diluted to 1X and 1/8X (instructions below)
- 1 1-liter and 1 2-liter soda bottle, with labels removed
- masking tape
- felt-tip marking pens
- 1 pack of dried bees (Cat. # BA-15-895)
- 1 box of toothpicks
- 1 tube of fast-drying Duco cement

The components for the potting mix and the Peter s Professional Fertilizer are available at most garden centers.

For each set of four groups of four students:

- 1 plant lighthouse (instructions below)
- 6 8-inch x 10-inch pieces of 1 inch thick rigid insulating foam

Sheets of insulating material can be purchased inexpensively from building supply stores. Your shop teacher may be able to cut up such a sheet for you, or you can cut it with a utility knife. Your students will use these six pieces of foam underneath their growth systems initially, so that when the seedlings emerge, they will be close enough to the light to grow vigorously. Then as their plants grow, students will remove the foam pieces one at a time, in order to keep the growing tips of the plants at the recommended distance from the light.

For each group of four students:

- 1 film-can growth system (instructions below)
- 30 F_1 seeds in a small envelope
- 1 water bottle (instructions below)
- 14 25-cm bamboo skewers (available in most supermarkets)
- 14 split-ring ties made from 9 or 10 mm O.D. Tygon or aquarium tubing (instructions below)
- 1 brown paper lunch bag
- 1 small envelope

For each group of four students in Phase 2:

- 1 envelope with 6 P_A seeds
- 1 envelope with 6 P_B seeds
- 1 envelope with 6 double-mutant seeds
- 1 envelope with the students own F_2 seeds

MATERIALS OPTION 2

Seeds required per 12 student groups (groups of four are recommended):

Catalog number	**No. of seeds**	**Phenotype**	**Genotype**	**Genetic role in exp.**
BA-15-8812	50	anthocyaninless (Anl, Ygr+)	*anl/anl, YGR/YGR*	P_A
BA-15-8818	50	yellow-green (Anl+, Ygr)	*ANL/ANL, ygr/ygr*	P_B
BA-15-8890	50	wild type (Anl+, Ygr+)	*anl/ANL, YGR/ygr*	F_1
BA-15-8888	250	segregating all four phenotypes	all of the above and below	F_2
BA-15-8842	50	anthocyaninless, yellow-green (Anl, Ygr)	*anl/anl, ygr/ygr*	double-mutant

For all classes combined:

- 3 liters of potting mix (1 part Peatlite, RediEarth, or JiffyMix and 1 part medium vermiculite)
- Peter s Professional Fertilizer 20-20-20 diluted to 1X and 1/8X (instructions below)
- masking tape
- felt-tip marking pens
- 1 1-liter and 1 2-liter soda bottle, with labels removed

The components for the potting mix and the Peter s Professional Fertilizer are available at most garden centers.

For each set of four groups of four students:
- 1 plant lighthouse (instructions below)
- 6 8 inch x 10 inch pieces of 1 inch thick rigid insulating foam

Sheets of insulating material can be purchased inexpensively from building supply stores. Your shop teacher may be able to cut up such a sheet for you, or you can cut it with a utility knife. Your students will use these six pieces of foam underneath their growth systems initially, so that when the seedlings emerge, they will be close enough to the light to grow vigorously. Then as their plants grow, students will remove the foam pieces one at a time, in order to keep the growing tips of the plants at the recommended distance from the light.

For each group of four students:
- 1 film-can growth system (instructions below)
- 1 envelope with 6 P_A seeds
- 1 envelope with 6 P_B seeds
- 1 envelope with 6 F_1 seeds
- 1 envelope with 6 double-mutant seeds
- 1 envelope with 18 F_2 seeds
- 1 water bottle (instructions below)

ADVANCE PREPARATIONS

Whether you use Option 1 or Option 2, the educational potential of the Fast Plants will be realized only if you provide the plants all of the resources they need to grow vigorously and fully express their genetic potentials. The most important resource is light. Fast Plants have been developed to grow rapidly and express their full potential under inexpensive fluorescent lights of the sort used in the home if — but only if — such light is provided at relatively high intensity and for 24 hours per day.

The lighting system that the Wisconsin Fast Plants Program currently recommends is a plant lighthouse (see below). The plant lighthouse has several significant advantages over the older light-bank system that was recommended previously*: It provides a more nearly ideal light intensity; it is both easier and cheaper to construct; and it takes up far less classroom space per plant grown. The plans given below are for making lighthouses that can be folded up and stored flat when they are not in use. They should remain useable for many years.

The other resources that your Fast Plants require are water, soil, and fertilizer. Information regarding simple, inexpensive ways of providing all these resources are also given below.

*Until rather recently, the lighting system that most teachers used was a set of 6 4-foot fluorescent tubes spaced 10 cm apart (as in the Carolina Biological Plant Light Bank kit, Cat. # BA-15-8998). If your school already has such a light bank, it may be used. Similarly, if your school has a collection of the watering systems and quad growing units purchased from Carolina for growing Fast Plants, they may be used. But in either case, please see the *Hints and Troubleshooting* section for ways to improve the performance of these components.

A. CONSTRUCTING A PLANT LIGHTHOUSE (FIG. 1)

Whether you are implementing Option 1 or Option 2, you will need one plant lighthouse for each four student groups.

Materials

For the entire project:
1 felt-tip marking pen
1 quarter
1 utility knife or other sharp, pointed cutting tool
1 or more glue sticks
1 roll of 12 inch-wide aluminum foil
1 pair of scissors
1 roll of clear, glossy tape

For each lighthouse:
1 empty copy-paper box (approximately 23 x 30 x 45 cm)
1 lid from a plastic container, 10-15 cm in diameter
1 foot of self-adhesive Velcro tape (hook and loop)
1 30 to 39 watt energy saver fluorescent circle-light with a socket adapter**
1 electric cord with a standard light socket on one end
1 wooden paint stirrer or other thin wooden strip approximately 30 cm long
1 20 x 26 cm piece of cardboard

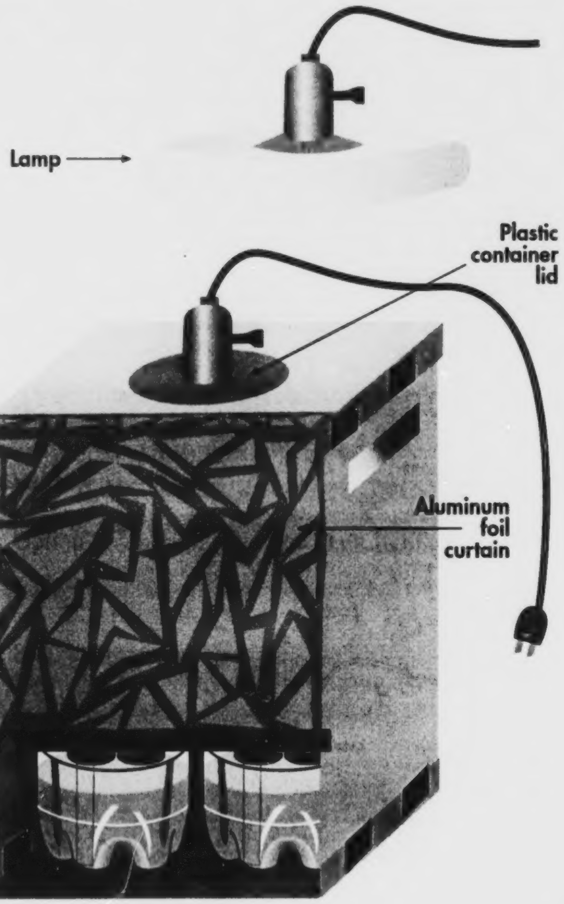


Figure 1

**A Lights of America 30 watt Circlight (model # 2630) or 32 watt light (model # 2730) is good; a GE 39 watt Energy Saver (Product Code 18739) is better. Such lights are sold at many discount and hardware stores. If you cannot find such a light locally, the 30 watt model is available (at considerably higher price) from Carolina Biological (Cat. # BA-15-8999 or BA-15-8997).

Construction Procedure

1. Discard the lid of the copy-paper box. Check the rest of the box carefully; if any flaps are loose, reglue them and allow the glue to dry.
2. Stand the box on end. Using the marking pen and the quarter, draw a circle in the middle of what is now the top of the box (Fig. 2) and on the plastic lid. Use the utility knife to cut out both circles carefully.
3. Make cuts 2.5 cm from each end, all around the box (Fig. 2), to separate both ends (A and C) from the rest of the box (B) (Fig. 3).
4. Lay piece B flat. Cut a 3 x 14 cm vent slot on each section of piece B 3 cm from the top edge (Fig. 4 B).
5. Attach 2.5 cm pieces of Velcro tape — loop half — to the outer surface of piece B in the locations shown (Fig. 4B).
6. Attach 2.5 cm pieces of Velcro tape — hook half — to the inner surfaces of pieces A and C (Fig. 4 A and C) in positions such that the hook pieces will make contact with the loop pieces on piece B when the box is reassembled (Fig. 5).
7. Apply glue stick to the inner surfaces of all three cardboard pieces, and cover all these surfaces with aluminum foil, shiny side up.

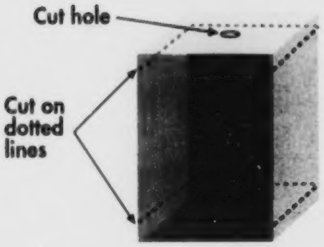

Cut hole
Cut on dotted lines

Figure 2

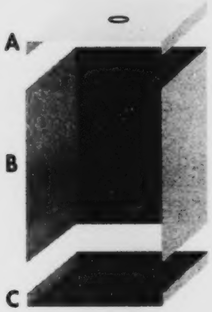


Figure 3

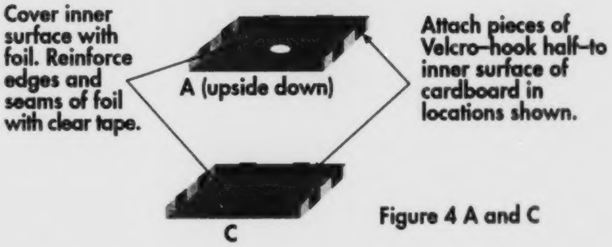

Cover inner surface with foil. Reinforce edges and seams of foil with clear tape.
A (upside down)
Attach pieces of Velcro–hook half–to inner surface of cardboard in locations shown.
C

Figure 4 A and C

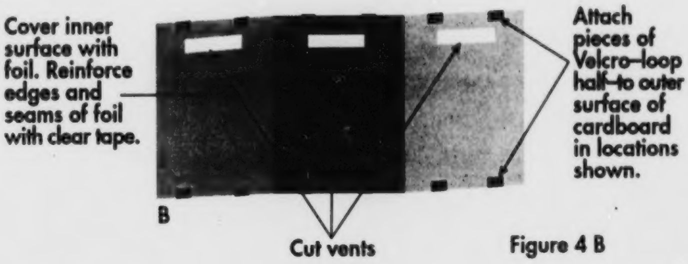

Cover inner surface with foil. Reinforce edges and seams of foil with clear tape.
Attach pieces of Velcro–loop half–to outer surface of cardboard in locations shown.
B
Cut vents

Figure 4 B

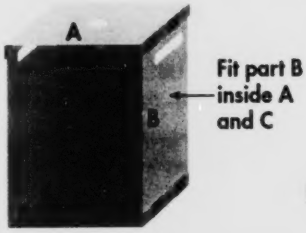

A
B
Fit part B inside A and C

Figure 5

8. Cut away the foil that covers the circular hole in piece A and the vent holes in piece B.
9. Use glossy clear tape to reinforce all the seams and edges of the foil, plus the bend regions of piece B.
10. Fold piece B and insert it into the end pieces, A and C, to reassemble the box (Fig. 5). Press Velcro tapes together to stabilize box.
11. Insert the lamp base through the hole in piece A from the inside. Slip the plastic lid with a hole in it over the lamp base. Screw the lamp into the socket (Fig. 1).
12. Cut a piece of aluminum foil 40 cm long. Reinforce the sides and middle of this foil with clear tape. Apply glue stick to both side of a paint stirrer, attach the stirrer to one end of the foil, then roll it over so that it is covered with foil on both sides.
13. Tape the other end of the foil (shiny side in) to the top of the box so that it forms a curtain over the top portion of the opening (Fig. 1). (It should not extend all the way to the bottom of the box.)
14. Cover the 20 x 26 cm piece of cardboard with foil, shiny side out. Reinforce the edges and seams of the foil with clear tape. (This piece of foil-covered cardboard will be placed between the plants and the foam blocks that prop up the plants.)

B. ASSEMBLING A FILM-CAN GROWTH SYSTEM (FIG. 6)

Whether you are implementing Option 1 or Option 2, you will need one film-can growth system for each group of four students. Four such growth systems are easily accommodated in one plant lighthouse.

Materials

For the entire project:
- 1 roll of narrow masking tape
- 1 can of quick-drying flat-black spray paint
- 1 4d finishing nail
- 1 pair of wire cutters
- 1 piece of wooden dowel ~10 cm long
- 1 drill with 5/64 and 3/16 drill bits
- 1 bunsen burner, alcohol burner, or other flame
- 1 pair of forceps
- 1 white correction pen or bottle of correction fluid

For each four student groups:
- 4 2-liter soda bottles
- 4 half-pound deli containers (Such containers are available at no cost to educators at many supermarket deli counters.)
- 4 1 x 36 cm strips of capillary mat (Capillary matting is available from horticultural supply companies, but often only in very large quantities. Pellon" from a fabric store will serve the same purpose; the heaviest available grade of Pellon is recommended.)
- 4 7.5 cm squares of capillary mat
- 4 1 x 8 cm strips of capillary mat

28 black 35 mm film cans (35 mm film cans are available in large quantities at no cost at most film-processing counters or kiosks.)

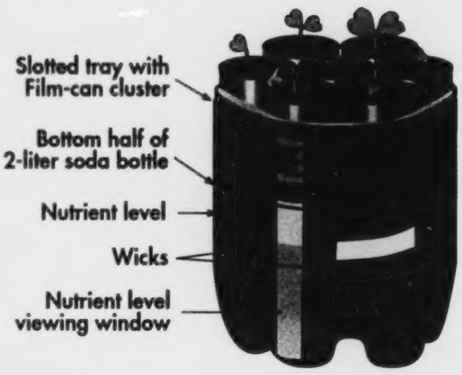
12 #64 rubber bands (#64 rubber bands are often used by mail carriers to bundle mail, and thus are available at no cost in many offices.)

20 cm of 9 or 10 mm O.D. Tygon or clear aquarium tubing

1 single-edge razor blade

Assembly Procedure

1. Nutrient reservoir. Peel the label off a 2-liter soda bottle after heating it with hot tap water or air from a hair dryer. Cut the bottle in two about 13 cm from the bottom. Discard the top piece and treat the bottom piece (which will become the nutrient reservoir) as follows:
   Attach a vertical strip of tape to one side. Spray the outside with several thin layers of flat black paint until it is nearly opaque. When the paint is dry, remove the tape to create an observation window (Fig. 6.). (The black paint is to suppress algae growth in the reservoir.)
2. A nail poke. Remove the head of the finishing nail with wire cutters. Drill a 5/64 inch hole in one end of the dowel. Force the blunt end of the nail into the hole (Fig. 7).
3. Slotted tray. Heat the nail poke in a flame and use it to melt two slots (2 x 12 mm) on opposite sides of the bottom of a half-pound deli container.


Slotted tray with Film-can cluster
Bottom half of 2-liter soda bottle
Nutrient level
Wicks
Nutrient level viewing window

Completed growth system
Figure 6

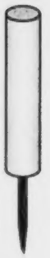
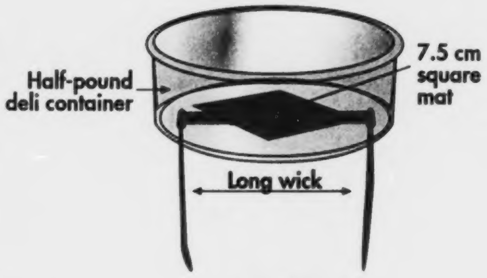


Figure 7


Half-pound deli container
7.5 cm square mat
Long wick

Slotted tray
Figure 8

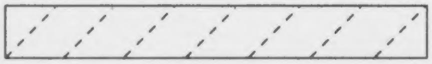


Figure 9

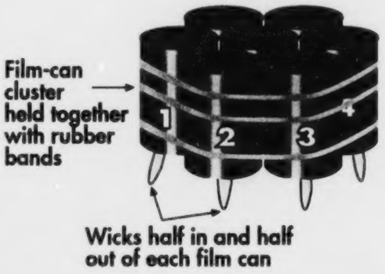

Film-can cluster held together with rubber bands
1
2
3
4
Wicks half in and half out of each film can

Figure 10

Thread a 1 x 36 cm piece of capillary mat through the two slots in the deli container so that it hangs down about the same distance on each side (Fig. 8). Place a 7.5 cm square of capillary mat in the bottom of the tray.

Place the slotted tray in the nutrient reservoir.

4. Film can cluster. Cut a 1 x 8 cm strip of capillary mat on a 45¡ angle to produce seven diamond-shaped wicks about 1 x 2 cm in size (Fig. 9).

   Drill a 3/16 inch hole in the bottom of each film can. Using forceps, insert one wick in the bottom of each film can, leaving about half of each wick protruding.

   Cluster seven film cans and secure them with two or three #64 rubber bands. With the correction pen or brush, number the six outer cans with numerals 1 through 6 (Fig. 10). The central can will be #7 but need not be marked.
5. Completed growing system. Place the film-can assembly into the slotted tray and place the slotted tray in the nutrient reservoir.

C. PREPARING THE POTTING MIX

Each film-can growth system requires about 250 ml of potting mix. Thus, for each planting you need about a liter of mix for every four student groups. Option 1 of the exercise requires two plantings (for the F_1 and F_2 generations), or about two liters of potting mix per four student groups. Option 2, which involves only one planting (the F_2 generation), requires only about one liter of potting mix per four groups.

Measure into a large pail or bucket equal volumes of a peat-based soilless planting formula (such as Peatlite, RediEarth, or JiffyMix) and medium vermiculite. Mix thoroughly by hand. Add water to moisten the mixture somewhat but not enough so that it clumps.

D. PREPARING NUTRIENT SOLUTION

You will need about 400 ml of 1/8 X Peter s Professional Fertilizer per film-can growth system.

Label a 1-liter soda bottle *1 X Peter s Stock Solution*. Add one soda-bottle capful of Peter s fertilizer (right out of the bag) to that bottle, add a liter of tap water and mix. Next, label a 2-liter soda bottle *1/8 X Peter s Nutrient*. Add 250 ml of water to the bottle, draw a line at the meniscus with a marking pen, and label the line *250 ml*. Pour out the water and pour in 1 X Peter s Stock Solution up to the 250 ml mark. Add 1750 ml of water. Two liters of 1/8 X Peter s Nutrient is adequate for four student groups, and more can quickly be made from the 1 X stock, as needed.

E. PREPARING WATER BOTTLES

Water bottles (one per student group) can be made from plastic soda bottles (16, 20, or 24 oz.) that have plastic caps. Heat the nail poke in a flame and melt a small hole in the cap. Fill the bottle with water, cap it, turn it upside down, and squeeze it to obtain a stream of water that will not disturb seeds or seedlings.

F. PREPARING SPLIT-RING TIES (NEEDED FOR OPTION 1 ONLY)

Use the single-edge razor blade to cut off 3 mm rings from the piece of Tygon tubing (also called *clear aquarium tubing*). Then use a pair of scissors to split each ring open, dropping finished rings into a container as you go.

OPTION 1 PROCEDURE

Detailed procedures are given on student pages S121-133.

Remember to (a) give the students only the Phase 1 instructions at the beginning, (b) have them plant only the F_1 seeds, and (c) refer to these seeds simply as Wisconsin Fast Plant seeds, because it would be premature to discuss any of the genetic aspects of the experiment at this time.

Seed-planting day for Phase 1 should be on either a Monday or Tuesday, so that the seedlings can be watered from above on three successive school days. Planting should take the students about 30 minutes.

Phase 1 Schedule

Well in advance: Construct plant lighthouses and film-can growth systems. If possible, test the growing system in advance for at least a week by following the student instructions.

Just in advance: Assemble all growing materials. In order to minimize the amount of clean-up, keep the potting mix in a single large container in a central location and have the students fill and level their film cans there. Provide a large spoon, a small spoon, and a ruler (or other straight edge) next to the potting mix.

Day 1 (on a Monday or Tuesday): Hand out the Phase 1 instructions, the film-can growing systems, and the F_1 seeds. Oversee the planting of the seeds.

Days 2-4: Remind students to water their film cans with their water bottles.

Every day: Have students check the nutrient levels in their reservoirs and add 1/8 X Peter s as necessary to keep the level up near (but not above) the bottom of the slotted tray. It is a good idea to add the 1/8 X Peter s to the film can tray and allow it to run through the slots to the reservoir; this will assure that the capillary matting remains saturated. Make sure that all reservoirs are full at the end of the day before weekends and holidays.

Days 4-7: Have students transplant some seedlings if necessary and/or remove extra seedlings, so as to end up with two healthy plants per film can.

Day 11 or 12: Have students assemble bee sticks.

Days 13-17: Have students cross-pollinate plants with open flowers on at least three successive days.

Days 17-35: Remind students to observe seed pod development at intervals. If time permits, you may want to dissect a few seeds at intervals, so students can view the embryos developing inside the seeds with a dissection microscope.

Day 35+ (approximately 20 days after last pollination): Have students remove their plants from the water so that the seed pods can dry out.

Day 38-40+: After the seed pods are thoroughly dry, have students harvest the seeds according to instructions on the student pages. Supply small envelopes for storing the seeds.

Phase 2 Schedule

Fast Plant seeds differ from most other seeds by not having any required dormant period before they will germinate. So after the seeds have been dried, you may begin the second phase of this experiment any time you wish. It is a good idea to purchase a supply of F_2 seeds (from Carolina Biological) just in case some or all of your students did not recover enough F_2 seeds to execute Phase 2 as outlined.

Remember that students did not get Phase 2 instructions initially, so give them to the students now.

It is important to begin Phase 2 on a Monday so that students will be able to observe their germinating seedlings daily for the first week. By the fifth day it should be possible to distinguish the phenotypes of all F_2 plants and record the results. Nevertheless, have students refill their water reservoirs before leaving for the weekend, so that they can recheck their results early in the following week.

You need to provide a mechanism for students to give a standardized name to the mutant phenotypes they will observe. There are at least three ways to do this:

¥ **Good**: You tell the class that the name for the mutant phenotype seen in the P_A seedlings is *anthocyaninless*, and that the name for the phenotype seen in the P_B seedlings is *yellow-green*.

¥ **Better**: You make color copies of the pictures of Fast Plant mutants in the Carolina Biological catalog and let the students use these to decide what phenotypes their plants exhibit. Alternatively, you can make your own Fast Plant photos as follows: When seedlings are 4 to 5 days old, snip off one seedling of each genotype at ground level. Use glue stick to attach the seedlings to a sheet of paper. Label each genotype. Quickly (before the plants wilt) copy the page on a color copier. This works surprisingly well.

- **Best**: If you did not grow a complete set of Fast Plant mutants at the beginning of the genetics section, (as suggested under *Hints and Troubleshooting*) do so now. Plant all of your mutant seeds (one type per film can) when the students are planting their F_2 seeds and label each type of mutant.

It is a good idea to check that each group can distinguish the anthocyaninless yellow-green (Anl/Ygr) double mutants from the two kinds of single mutants. This distinction is important in determining whether their F_2 plants behaved according to the expectations of Mendelian genetics.

You also need to provide a mechanism for combining and tabulating the class data. A good way of doing this is to use a strip of butcher paper to prepare enlarged versions of Tables 1.B and 2.B that have columns in which all groups can enter their data, plus a column for class totals.

OPTION 2 PROCEDURE

Detailed procedures are given on student pages S141-145. Note that the work sheets for Option 1 Phase 2 are also to be used for Option 2. However, they have not been duplicated at the end of the Option 2 student pages.

It is important to begin this experiment on a Monday, so that students can to observe their germinating seedlings daily for the first week. By the fifth day it should be possible to distinguish the phenotypes of all F_2 plants and record the results. Nevertheless, have students refill their water reservoirs before leaving for the weekend, so that they can recheck their results early in the following week.

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HINTS AND TROUBLESHOOTING (BOTH OPTIONS)

1. The Wisconsin Fast Plants can help you dramatize the concept of heritable variation in visible traits at the very beginning of the genetics unit.

   If you have space available in a plant lighthouse two weeks before you begin the genetics unit, consider setting up a growth system with a different variant in each film can. If you are doing Option 1, your students Fast Plants should be nearly mature (and wild-type in phenotype) by that time. Thus, they should be able to recognize the various phenotypic differences quickly and easily. This would be a good time to let them in on the big secret: namely, that even though all of the plants they have been growing appear to be normal (wild-type), they are actually carrying invisible versions of two of the mutant traits visible in your seedlings. (But even Option 2 students should be able to see the visible differences among these plants and become intrigued by their heritable basis.)

   In short, you can use the Fast Plants at the beginning of the genetics section to stimulate student interest in understanding how visible traits are controlled, hidden from view in one generation, and then caused to reappear in another generation.

   If you decide to go this route, you should already have seeds of four distinctive genotypes to plant in four of the film cans: namely, the P_A (Anl), P_B (Ygr), F_1(wild-type) and the double mutant seeds (Anl/Ygr). Additional mutant seeds you should consider purchasing for use in the other three film cans include rosette (Cat. # BA-15-8815), elongated internode (Cat. # BA-15-8824) and AstroPlants (Cat. # BA-15-8835).

   If you cannot grow a complete set of demonstration mutants before the beginning of the genetics section, consider doing so when the students are starting Phase 2 (or Option 2) of the exercise.

2. On the page where the construction of a plant lighthouse was introduced, it was noted that if you already have a six-tube fluorescent light bank (such as Carolina Biological Cat. # BA-15-8998) you may grow the Fast Plants under it, even though its light output is on the marginal side. (A four-tube bank of this sort does not provide adequate light, however, and should NOT be used.) Growth under a six-tube bank is improved if (a) the fluorescent tubes are replaced every two years, (b) aluminum foil curtains

(weighted at the bottom) are attached to all four sides of the light bank, and (c) adjustable supports are placed under the plants to keep their growing tips no more than 10 cm from the lights.

It was also noted that if you already have a collection of the styrofoam quads and other growth system components that Carolina Biological sells for growing Fast Plants, you may use them instead of the film-can growth system described in this unit. However, you should consider setting up at least one film-can growth system for comparison purposes. You might even want to ask one student group to set up both kinds of systems and to make careful comparisons of plant performance in the two systems.

3. A follow-up exercise — or a couple of potential science fair projects. The focus of the Fast Plant experiments so far has been on the two recessive single-gene traits that distinguish the PA and PB plants and members of the F_2 generation. However, there is another trait that distinguishes the PA and PB plants. It appears before the seedlings are a week old and becomes very clear by the time the plants are eight to ten days old. Challenge your students to figure out what it is. They will need to use good illumination and a good hand lens to see this PA-PB difference — and even then they may have great trouble seeing it. But once they figure out what the trait is, it will jump out at them! By the way, an eyepiece from a microscope, used backwards, makes an excellent hand lens for use in such studies.

   The answer (which students should be allowed to discover for themselves) is that the PA (Anl) plants are almost completely hairless, whereas the PB (Ygr) plants are hairy ; that is (like most plants), they have many hairlike outgrowths on their leaves and stems (Fig. 11).

   The function of such plant hairs is not yet fully understood, but it is thought that they serve such purposes as deterring insect predators, interfering with wind flow (thereby decreasing the rate of water loss from leaves), and increasing the efficiency of light absorption.

   Hairiness is not an all-or-none trait. It is a **quantitative variable**. That is, plants differ widely in the degree to which they express the trait. The standard designator for the hairy phenotype is Hir, (from *hirsute*, the Latin word for *hairy*), and Fast Plants range from Hir(0) (completely hairless) to Hir(9) (extremely hairy). Your students PA (Anl) plants are rated Hir(1), and their PB (Ygr) plants are rated Hir(5).

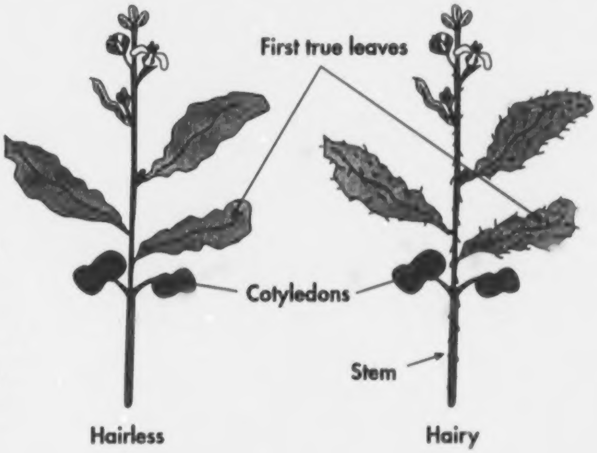


Figure 11

Below are a few of the simpler questions that you might pose to your students, and some sample answers that they should come up with if they merely observe their plants carefully.

Q: Does the hairy trait disappear in the F_1 generation like the Anl and Ygr traits do?
A: No. The F_1 plants are somewhere in between the two parental types in hairiness.

Q: What does this say about the heritable basis of the hairy trait?
A: It appears to be a heritable trait that exhibits co-dominance, or incomplete dominance.

Q: How does the hairiness of the F_1 plants compare with the hairiness of the F_2 plants?
A: The F_2 plants are much more variable in their hairiness than the F_1 plants.

Q: Does hairiness cosegregate with Ygr in the F_2 generation? (That is to say, are the Ygr plants routinely hairier than the Anl plants in the F_2 generation, as they are in the parental generation?)
A: No. The hairy and yellow-green traits appear to be inherited independently.

Q: Do the F_2 plants fall into only three classes that resemble the PA, PB, and F_1 plants with respect to number of hairs?

A: No. The F_2 plants are much more variable in hairiness than that.

Q: What does this imply about the heritable basis of the hairy trait?
A: Hairiness probably is controlled by genes at more than one *hairy* locus. (If the PA and PB plants differed by only one allele at one locus, then the F_2 plants should fall into three distinct classes representing the heterozygote and the two homozygotes; but careful inspection will reveal that they do not.)

Q: How can you rule out the possibility that the variation you see in the F_2 plants is caused by environmental variables that you were not aware of and could not control?
A: The PA, PB, and F_1 plants provide controls that appear to rule out that possibility, because they all exhibit much less variability in hairiness than the F_2 plants do. If there were uncontrolled environmental variables, there is no reason that they should affect only the F_2 plants.

Below are some questions of a quantitative nature that you might pose to your students. To answer these questions, students would have to count hairs and perform various statistical tests.

Q: Are the number of hairs on one part of a hairy plant (such as the edge of the first true leaf) correlated with the number of hairs on another part (such as the surface of that leaf or the edge of the second leaf)?

A: Yes. But obtaining this answer probably would be a highly instructive statistical-analysis exercise. something with which the math teacher might help.

Q: How much variation is there in the number of hairs on equivalent structures (such as the first true leaves) in a group of plants that are presumably similar genetically (such as the P_B plants)?

A: There is no single or simple answer to this question, but it could lead a motivated student into an extended study of natural variability and the way that such variability is evaluated statistically.

There are other interesting questions that you might pose to your students. These questions could be answered only by performing additional crosses between their various plants and/or growing additional plants of known genotype under different conditions. Some of these questions could be used for great science fair projects. Just a couple of examples:

Q: How would you perform a scientific test of your hypothesis (mentioned above) that the hairy trait is controlled by genes at more than one locus?

A: The best way to test this hypothesis would be to separate out the F_2 plants with the least hairs and the most hairs, cross-pollinate each kind with another plant of the same type, and see how hairy their F_3 offspring turn out to be. If the parental plants differ with respect to only one locus that influences hairiness, then both sets of F_3 plants should have the same range of hairiness as the F_2 generation did. But if there are multiple hairy genes, then the very hairy F_2 plants should produce very hairy F_3 offspring, and vice versa. Repeating this kind of selective breeding for another generation or two could provide a great study of the effect of selection on plant evolution.

Q: How could you test the hypothesis that the number of hairs produced by a plant is controlled by both its genotype and the environment to which it is exposed during development?

A: The best way to test this hypothesis would be to grow plants of two different but fairly uniform genotypes (such as the P_B and F_1 plants) under a range of environmental conditions and see how these conditions affect the number of hairs produced. Environmental factors to be tested might include such things as light intensity, or light color, and the concentration of fertilizer and/or salt in the nutrient reservoir.

ANSWERS TO THE QUESTIONS ON THE WISCONSIN FAST PLANTS WORK SHEET STUDENT PAGES 137-140

1. What are the two mutant traits that distinguished your PA and PB plants from one another and from wild-type Wisconsin Fast Plants?
The PA plants are anthocyaninless mutants.
The PB plants are yellow-green mutants.

2. If the mutant traits exhibited by the PA and PB generation are heritable, why didn t those two traits appear in their progeny in the F_1 generation?
They are both recessive traits that are not expressed in the heterozygous F_1 plants.

3. Based on your explanation above, what would you predict that the ratio of wild-type to mutant individuals for each of these two traits in the F_2 generation? Explain.
3:1 The probability that an individual will receive a recessive allele from each of its heterozygous parents is one in four. Thus, 1/4 of the offspring should have the recessive phenotype.

4. Above each of the tables below, record how many F_2 plants germinated and grew large enough that their phenotypes could be determined with confidence. Then in the right hand column of each table record how many of these F_2 plants had each of the indicated phenotypes.
All groups should have different data in Table 1.A, but the same data in Table 1.B.

5-7. With respect to the PA trait, how does the ratio of wild-type to mutant individuals that you predicted in Question 3 compare to the ratios of wild-type to mutant individuals that you reported in tables 1 A and B?
The predicted wild-type-to-mutant ratio was (in each case) 3:1

All groups should have different ratios for their own plants, but the same ratio for the class plants.

It should be very interesting to see how your students decide what constitutes a significant difference between predicted and observed ratios, and how much confidence they have in their ability to make such judgments.

This should provide you with an excellent opportunity to discuss the role of statistical analysis (as opposed to gut feelings) in making such decisions with respect to scientific observations.

8. Record the observed phenotypes of the F_2 plants with respect to combinations of PA and PB traits.
Again, all groups should have different data for their own plants, but identical data for the class.

9. In the table below, compare the ratios of the four possible combinations of PA and PB traits that you and your class observed with the ratios that are predicted for this kind of dihybrid cross. In each case, set the number of double-mutant plants to one.
The predicted ratio is 9:3:3:1, but all groups should have different ratios for their own data and identical ratios for the whole class.

10-11. Are the differences between the predicted and the observed ratios in the above table significant?
Again, it should be very interesting to see how your students decide what constitutes a significant difference between predicted and observed ratios, and how much confidence they have in their ability to make such judgments.

If, for some reason, you have decided not to make unit 2.E a class assignment, encourage your students to study it voluntarily, to find out about one widely used method of deciding whether differences between predicted and observed experimental results are significant.

REFERENCES

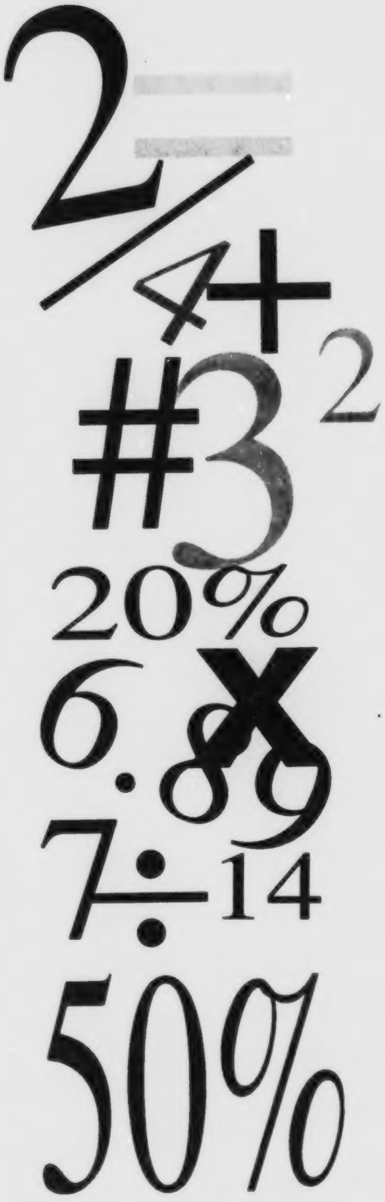
1. *Catch the Spiral: Cycle Through Life With Fast Plants* (2000). (ISBN 0-7872-6910-7) Kendall/Hunt Publishing Company, Dubuque, Iowa. Tel. 800-228-0810; Fax 800-772-9165; www.kendallhunt.com 25 explorations in plant biology. Developed by Dr.. Paul H. Williams and other members of the Wisconsin Fast Plants Program, College of Agriculture and Life Sciences, University of Wisconsin-Madison.

2. *CrGC Information Catalogue*(1997). Crucifer Genetics Cooperative, University of Wisconsin-Madison, Madison Wis. Tel. 608-263-2634; Fax 608-263-2626; www.fastplants.cals.wisc.edu/crgc/crgc.html This catalog and information brochure from the Crucifer Genetics Cooperative (which is closely associated with the Wisconsin Fast Plants Program) lists seeds for a much wider variety of *B. rapa* mutants, as well as several other species of crucifers.

3. *Wisconsin Fast Plants Poster.* Carolina Biological. Tel. 800-227-1150; www.carolina.com This colorful 24 x 36 poster illustrates the rapid development and complete life cycle of the Fast Plants.

ACKNOWLEDGMENTS

We gratefully acknowledge the assistance of the Wisconsin Fast Plants Program in preparing this exercise. The illustrations in this exercise were adapted, with permission, from ones used by the Wisconsin Fast Plants Program in teacher workshops.



CHAPTER 2

Passing Traits from One Generation to the Next

SECTION E

How Are Genetic Results Evaluated Statistically?

Chapter 2: Section E Background

MANY BIOLOGY STUDENTS (and not a few biology teachers!) suffer "math insecurity." Many of them probably would just as soon go through life not thinking about any mathematical issues more complicated than counting their change at the pizza parlor. However, being able to make quantitative judgments, in addition to qualitative ones, is an important part of being a scientifically literate, responsible citizen. Obviously, you cannot be expected to completely counteract the math insecurities of all of your students and turn them all into enthusiastic statistical whiz kids while teaching them genetics. But you can conceivably help lower the barriers to thinking quantitatively outside of the math classroom, if you approach this exercise as a fun and easy way – rather than a painful but necessary way – to determine whether all the time and effort that they put into the Fast Plants experiment yielded results consistent with Mendel's "laws" of heredity!

E.1

Introduction to Using Statistics to Evaluate Genetic Explanations

STUDENT PAGES 148-149

LESSON OVERVIEW

This lesson is a reading assignment to prepare your students to work with the concepts behind a Chi square analysis. It introduces them to the problem of evaluating genetic results statistically with a "real life" situation that they should have no problem identifying with.

E.2

Too Many White Kittens? Using Chi Square (χ^2) to Find Out

STUDENT PAGES 150-151

LESSON OVERVIEW

This is a guided practice activity on working with the Chi square analysis formula. The students will learn the concepts behind each step of the Chi square process in a non-threatening exercise.

TIMELINE

It will take an average student about 15 minutes to read sections E.1 and E.2 carefully enough to understand the concepts involved.

E.3

How to Perform a Chi-Square Test on Any Data Set STUDENT PAGES 152-155

LESSON OVERVIEW

This is a guided practice activity on working with the Chi square analysis formula. The students will learn the concepts behind each step of the Chi square process.

TIMELINE

Assuming that the Fast Plant data for the class have already been tabulated and made available, calculation of the χ^2 and p values should not require more than 15-20 minutes.

ANSWERS TO THE QUESTIONS ON THE WISCONSIN FAST PLANTS™ CHI SQUARE WORKSHEET

There will be two sets of correct answers for the first Chi square analysis, but all students should get the same result for the second Chi square analysis. If you are going to have the students perform the analyses in class, it would be a good idea to work out the correct answers for yourself in advance, once you have the class data. Blanks are left below for this purpose.

Part One, A Simple Trial Run

The mutant trait I am analyzing by Chi square is *anthocyaninless*

Total number of F_2 plants obtained by the class: _________.
Multiply the total number of F_2 plants by the expected frequencies (3/4 and 1/4) to get the expected numbers of plants in each category. You probably will get non-integral numbers.

Phenotype	Expected #	Observed #	Difference	Difference^2
Wild-type				
Mutant				

$$\chi^2 = \frac{(\text{Observed - Expected})^2}{\text{Expected}} \text{ summed for all classes}$$

(Remember: "classes" here refers to classes of plant phenotypes, not classes of students!)

χ^2: _______ p___________ Use the table on page S143 to determine the value of p.

The mutant trait I am analyzing by Chi square is *yellow-green*

Total number of F_2 plants obtained by the class: _________.

Phenotype	**Expected #**	**Observed #**	**Difference**	**Difference^2**
Wild-type				
Mutant				

Again multiple the total number of F_2 plants by the expected frequencies to get the expected numbers of plants in each category. You may get non-integral numbers.

$$\chi^2 = \frac{(\text{Observed - Expected})^2}{\text{Expected}} \text{ summed for all classes}$$

χ^2: _______ p___________

Conclusion: *Presumably both Chi square analyses in part one will indicate that the data are consistent with the hypothesis that the mutant trait in question is due to a recessive allele at a single locus (which we know on the basis of prior studies is the case for both traits). If your advance analysis indicates that one or both of the Chi square analyses do not lead to this conclusion, you will need to be prepared to lead a discussion of alternative hypotheses. (One of which might be that certain groups failed to classify certain of their plants correctly!)*

Part Two, Chi Square Analysis of the Two Phenotypes at Once

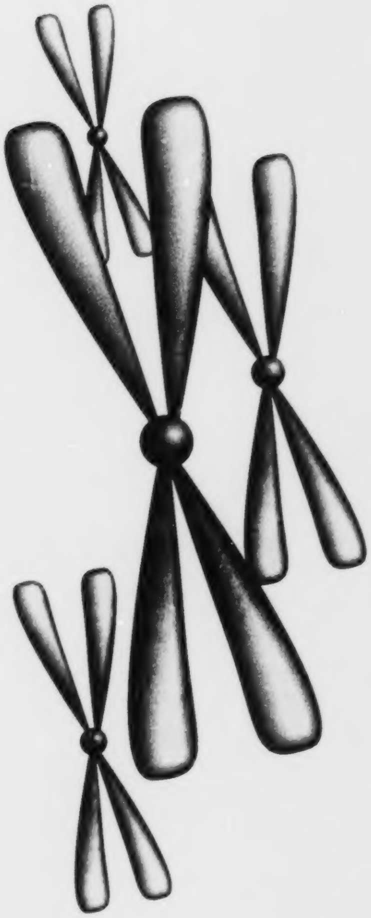
COMBINED DATA FOR THE ENTIRE CLASS **TOTAL NUMBER OF F_2 PLANTS: _____**

'a' phenotype	'b' phenotype	Frequency expected*	Number expected	Number observed	Difference	Difference^2
Wild-type	Wild-type					
Mutant (aa)	Wild-type					
Wild-type	Mutant (bb)					
Mutant (aa)	Mutant (bb)					

*You can obtain these frequencies by either (a) using the product-of-probabilities method, (b) using a Punnett Square, or (c) reviewing similar calculations you made for previous exercises.

χ^2: ________ p___________

Conclusion: *Presumably the Chi square analysis here will indicate that the data are consistent with the hypothesis that the 'a' and 'b' loci assort independently. Once again, if your advance Chi square analysis does not lead to this conclusion, you will need to be prepared to lead a discussion of alternative hypotheses. (One of which might be, once again that certain groups failed to classify certain of their plants correctly!)*



CHAPTER 3

How Genes and the Environment Influence Our Health

CHAPTER 3

How Genes and the Environment Influence Our Health

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CHAPTER 3

How Genes and the Environment Influence Our Health

SECTION A

How Stable and How Powerful is DNA?

A.1

DNA Paradoxes

DNA IS A MOLECULE of many apparent contradictions.

The continuity of life from generation to generation depends upon the fact that DNA molecules are usually extremely stable and are passed on unchanged from one generation to the next. But at the same time, the ability of life to change and adapt to new circumstances depends on the ability of DNA to undergo rapid changes from one stable state to a new stable state. The constancy of most of our DNA accounts for the fact that we and other people around the world are recognizable as members of the same species. Yet stable variations in human DNA that have accumulated through the ages account for the fact that we all have recognizable differences. Extending this concept to other organisms, DNA is the source of both the striking similarities and the amazing differences among the many life forms that populate our planet.

We may tend to think of DNA as being all powerful, because it can store instructions for making all the parts of all the organisms on earth. Yet in a very real sense DNA is totally powerless. A slave to its environment, DNA is unable to mediate any of the important reactions of life. It is even dependent on many other kinds of molecules that are present with it inside a cell for one of the most important reactions of life, its own replication.

Furthermore, it is the environment inside and around the cell in which a gene exists, and not the gene itself, that determines whether any of the information that the gene carries will ever be used, and if so, when, how, and with what effects.

It takes thousands and thousands of gene products all working together smoothly to keep us in good health. But a single gene product that is unable to function properly can be enough to destroy our health.

In this chapter, you will deal with some of these apparently contradictory aspects and complexities of DNA biology. First, you will perform experiments to study how different aspects of the environment interact to determine whether or not DNA will undergo a permanent change, or **mutation.** Then, you will take two different approaches to examining how certain aspects of the environment can determine whether or not particular genes that are part of an individual's genotype will be expressed to the extent that they will significantly influence the individual's phenotype. This will lead into an extended study of the roles that particular gene mutations play in human health. The culmination of this section will come when you prepare a brochure designed to help a genetic counselor provide an affected family with useful information about a genetic disease that affects one or more members of that family. Then, in the last exercise of this section, you will use a technique called **gel electrophoresis** to simulate the process by which a genetic counselor would determine which members of the family might be likely to suffer symptoms of the heritable disease in question.

CHAPTER 3

How Genes and the Environment Influence Our Health

SECTION B

How Do Heritable Changes in Genes Occur?

B.1

Inducing Mutations with Ultraviolet Light

INTRODUCTION

LIFE DEPENDS ON THE stability of DNA molecules and the ability of the cell to replicate them accurately generation after generation. But occasionally a DNA molecule is damaged by something in the environment. And occasionally mistakes occur during DNA replication. Either type of accident can result in a mutation, a heritable change in a gene. A DNA molecule that has been changed by mutation is normally just as stable and just as capable of faithful replication as it was before the mutation. Therefore, mutations can be passed on. The human genetic diseases you will study later in this chapter all involve mutations.

Some mutations cause no detectable change in the organism in which they occur, and some cause only unimportant changes. But many mutations cause serious abnormalities or death. Occasionally, however, a mutation occurs that improves the adaptation of an organism to its environment. It has been the slow accumulation of such rare, beneficial mutations in different kinds of organisms that has resulted in the diversity of the living world. When a mutation occurs in the absence of any human intervention, it is said to be **spontaneous** (even though it may have been the result of some external influence). But when it occurs as a result of a deliberate act of a biologist (usually as part of a genetic study), it is called an **induced mutation.** In this exercise, you will attempt to induce mutations that cause bacteria to change color.

You will use **ultraviolet light** (UV) to induce mutations in your bacteria. UV is present in sunlight as radiation that (as the name suggests) is just beyond the violet end of the visible spectrum. UV is absorbed by, and damages, DNA, and therefore it is a powerful **mutagen** (mutation-causing agent) and **carcinogen** (cancer-causing agent). Fortunately, however, the ozone layer of earth's upper atmosphere filters out most of the UV that streams toward earth from the sun. If the ozone layer were to disappear, the surface of the earth would become uninhabitable. Indeed, some scientists believe that the drastic decline in frog and salamander populations that has been observed around the world in the past ten years is probably a result of the thinning of the ozone layer. It is also believed that this thinning has been caused by chlorofluorocarbons (CFCs), such as Freon, which were once used as refrigerants and propellants in hair-spray cans.

Even at present ozone levels, the ability of organisms to live at the surface of the earth is due to their remarkable ability to repair DNA damage that has been caused by UV. The importance of DNA repair is highlighted by a genetic disease called xeroderma pigmentosum (XP) in which a critical enzyme required for DNA repair is nonfunctional. People with XP characteristically have many skin abnormalities, and most of them die of skin cancer before age 20.

As important as they are, however, our DNA repair systems are inadequate to fully protect us against the carcinogenic effects of the UV rays in sunlight or tanning salons. It is now recognized that a single sunburn as a child or teenager can increase the risk of skin cancer many years later. Those who deliberately expose themselves to UV by sun-bathing or visiting tanning salons are playing a dangerous game of chance somewhat similar to Russian roulette.

Because UV is absorbed by the top few layers of skin cells in humans, however, UV radiation cannot penetrate to our ovaries or testes, where our germ cells are stored. Thus, UV damage in humans is largely restricted to cells in the skin and eyes. Although it is the principal cause of human skin cancer, UV does not cause heritable mutations that can be passed on to our offspring.

In contrast, small one-cell organisms, such as bacteria, do not have the benefit of a protective layer of skin, and any nonfatal UV-induced mutations that they experience are invariably passed on to their progeny. We will take advantage of that fact in this exercise to study the mutagenic effects of UV on bacteria. We will also study the effects of visible light on bacteria that have been exposed to UV.

Serratia marcescens, the bacterium you will use for this experiment, normally produces a red pigment. Synthesis of the pigment involves many different enzyme-catalyzed steps and can be blocked by a mutation in any one of the genes encoding the numerous enzymes required for pigment synthesis. Therefore, pigment synthesis by *Serratia* provides a sensitive test for the presence of mutagenic agents. It is important to note that bacteria normally are haploid (have only one copy of each gene); therefore there are no recessive mutations in bacteria, and every mutation normally exerts its full effect right away. In this exercise, we will subject *Serratia* to **UV mutagenesis,** the induction of mutations with UV.

As a way of studying the effects of visible light on UV-exposed bacteria, your teacher will divide your class into two sets of groups. One group will incubate its UV-irradiated bacteria in the dark, and the other group will incubate its UV-irradiated bacteria in the light.

MATERIALS

For each group of four students:
- 1 marking pen
- 4 petri dishes containing agar
- 1 tube containing *Serratia* bacteria
- 1 1000 µl micropipettor
- 1 sterile pipette tip
- 1 sterile inoculating loop
- 1 UV light source
- 1 watch with second hand
- plastic wrap (light-incubated groups only)
- aluminum foil (dark-incubated groups only)
- 1 spray bottle of disinfectant

PROCEDURE

Day 1: Irradiating the Bacteria

1. With the marking pen, label the bottom of each dish with your group name, either *light* or *dark* (depending on which group you have been assigned to) and one of the UV exposure times: 0, 30, 60, or 90 seconds (Fig. 1).

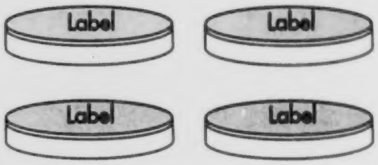


Figure 1

2. Swirl the tube of bacteria to suspend them evenly. Use the micropipettor to add 100 µl of bacteria to the middle of each dish (Fig. 2). Measure carefully so that each dish will have the same number of bacteria.

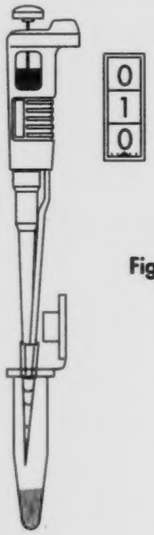


Figure 2

3. Use a sterile inoculating loop to spread the bacteria over the entire surface of each dish (Fig. 3). Avoid rubbing hard enough to damage the agar, but spread the bacterial culture as uniformly as possible. Place the inoculating loop in the waste beaker after use.

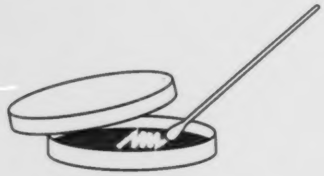


Figure 3

4. Take your plates to the UV light when directed by your teacher. WARNING: UV light can damage your eyes! Do not look into the light when it is turned on.

5. Place all four dishes with their lids on directly below the UV light. (UV light does not pass through the lids of petri dishes, so the bacteria will only be UV irradiated when the lids are off the plates.)

   Make one member of your group responsible for timing the UV exposures. When that person gives the signal, remove the lids from all three dishes that are to be irradiated. (Do NOT remove the lid from the 0 sec. control dish.) When the person doing the timing says, "30 seconds," cover the dish marked *30 sec.* Continue until the other two dishes have been exposed for 60 and 90 seconds.

6. If you are in a "light" group, wrap your dishes in plastic wrap. Then take all four dishes to the well-lit area indicated by your teacher and leave them there to incubate.

   If you are in a "dark" group, stack all four dishes, wrap them tightly in aluminum foil, and label the foil with your group name. Place the stack where your teacher indicates.

7. Wipe your work area with disinfectant. Wash your hands.

8. Allow the bacteria to grow at room temperature for 3-4 days.

Day 4 or 5: Here Come the Mutants!

1. If yours is a "light" group, team up with a "dark" group, and vice versa. Unwrap the dark-incubated dishes and lay out the light- and dark-incubated dishes side by side.

2. Observe the number, size, and colors of the bacterial colonies on each dish. Make a representative drawing of the dish on the UV Mutagenesis work sheet. You do not need to count the exact number of colonies on each dish, but summarize your observations in the spaces provided.

3. When you have finished describing your observations, open each dish, spray the agar surface with disinfectant, tape the dishes shut and dispose of them as your teacher instructs you to do. Wipe your work area with disinfectant. Wash your hands.

4. Answer the questions on the work sheet.

B.1

Name ______________________________

Date ____________________ Hour __________

UV MUTAGENESIS WORK SHEET

1. Draw pictures of what you observe on your dishes:

Dark-incubated dishes

0 Seconds　30 Seconds　60 Seconds　90 Seconds

Light-incubated dishes

0 Seconds　30 Seconds　60 Seconds　90 Seconds

2. Describe the bacterial colonies on your dark-incubated control dish (0 seconds of UV). Then list and describe the differences among the bacterial colonies seen on each of the dark-incubated experimental dishes relative to those on the dark control dishes.

   Dark-incubated control (0 sec.) dish: ______________________________

   Dark-incubated 30 sec. dish ______________________________

   Dark-incubated 60 sec. dish ______________________________

   Dark-incubated 90 sec. dish ______________________________

3. Based on these observations, summarize the effects that UV irradiation has on *Serratia* bacteria.

B.1

Name ______________________________

Date ____________________ Hour __________

4. Now describe any differences among the bacterial colonies on each of the light-incubated dishes relative to those on the corresponding dark-incubated dish. (That is, compare the light-incubated control dish to the dark-incubated control dish, the light-incubated 30 sec. dish to the dark-incubated 30 sec. dish, and so forth.)

   Control (0 sec.) dishes: ______________________________

   30 sec. dishes: ______________________________

   60 sec. dishes: ______________________________

   90 sec. dishes: ______________________________

5. Based on the above observations, summarize the effects that cultivation in visible light has on UV-exposed *Serratia* bacteria.

6. Can you formulate a hypothesis to account for such an effect of visible light?

7. Do all of the bacteria on a dish appear to respond to UV and visible light in the same way? Why?

8. Do all of the mutations that you observe in *Serratia* after UV irradiation appear to be harmful? Explain.

9. Is it possible that a mutation could be beneficial? Explain.

CHAPTER 3

How Genes and the Environment Influence Our Health

SECTION C

Is it Nature, or Is it Nurture?

C.1

Albino Plants: A Model Gene-Environment Interaction

INTRODUCTION

WHETHER THE TOPIC HAS been bird migration, human intelligence, cancer, or any one of countless other biological phenomena, nonscientists – and occasionally some scientists – have often argued endlessly about whether the explanation for the phenomenon should be sought in **nature** (genetic differences) or **nurture** (environmental influences). To most biologists such arguments constitute just so much wasted breath. This is because they recognize one overriding generalization about the living world to which there are no known exceptions. And if you remember nothing else from your study of genetics, this is the generalization that you should remember: **Every phenotypic trait of every living being is the result of some set of gene-environment interactions.** If one wants to understand any particular trait of any individual organism, human or otherwise, the question to be answered is not whether that trait has a genetic or an environmental basis. The question to be answered is what genes and what environmental factors have interacted to shape that trait.

In this section, we will take two approaches to exploring this concept. The first will involve a lab experiment, in which we will examine the way in which genes and environment interact during plant development to control the production of **chlorophyll,** the green pigment required for photosynthesis. The second will involve a simulation, in which we will explore how genetic and environmental factors interact to determine one's prospects of developing heart disease, the principle cause of death in the United States and most other industrialized countries.

When we look around a country hillside or a city park in late spring, we see such a lush abundance of green trees, green grass – yes, and green weeds – that we tend to take for granted the chlorophyll molecule that paints our surroundings green. We may reflect on the fact that our own life, and the life of all other organisms around us, depends on chlorophyll, the wonder molecule that captures the energy of sunlight and makes photosynthesis, and this life, possible. But probably no one but a plant biologist ever spends much time worrying about how the synthesis of this "green" molecule is controlled. Leaves are green, and that's that, right?

In this exercise, however, we will get vivid evidence that chlorophyll synthesis is a clear example of the generalization that all traits of all organisms are a result of gene-environment interactions. To be more specific, we will germinate seeds produced by tobacco plants that were heterozygous for a gene that has a dramatic effect on chlorophyll synthesis. Half of the class will allow their seeds to germinate in the light, while the other half will place their seeds in a dark place to germinate. This will permit your class to determine how an environmental factor, light, interacts with the genotype of the seedlings to regulate chlorophyll production.

C.1

MATERIALS

For each group of students:
- 1 petri dish containing black agar
- 1 marking pen
- 30 tobacco seeds

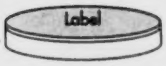


Figure 1

PROCEDURE

Your teacher will divide the class into two sets of groups, designated "light" and "dark," and will also indicate which "light" and "dark" groups will pair up for the data analysis.

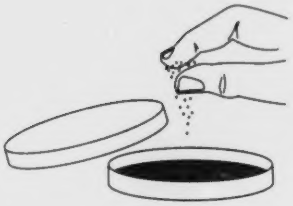


Figure 2

1. With the marking pen, label the bottom of your Petri dish with your group name, hour, and either light or dark, depending on which team you were assigned to by your teacher (fig 1).

2. Count out 30 tobacco seeds. Make a small pile with the seeds and press your index finger down onto them. The seeds should stick to your finger. Raise the lid of your petri dish and use your thumb to gently rub the seeds from your finger; sprinkle the seeds evenly over the surface of the agar (fig. 2). Do not touch the agar or leave the dish uncovered any longer than necessary.

3. If you are in a "light" group, place your petri dish under the lights. If you are in a "dark" group, wrap your petri dish in foil before placing it under the lights. Write the name of your group on the foil.

4. When your teacher says it is time to do so (which will probably be 7-10 days later), observe your seedlings. Count and record the number of green seedlings and the number of white seedlings on your plate. Record the numbers on your Albino Plant work sheet. If you are in a "dark" group, share your data with your partner "light" group and vice versa. Record the data collected by your partner group on your work sheet.

5. Whether you are in a "light" or a "dark" group, place your petri dish under the lights for further growth. (Do not wrap the "dark" dishes in foil this time.)

6. When your teacher says it is time to do so (which will probably be 2-3 days after the first observation), observe your seedlings and the seedlings of the other group with which you are paired. Count and record the number of green seedlings and the number of white seedlings on both dishes and record the numbers on your Albino Plant work sheet.

7. Record the data obtained by your two groups on the class data table. When the numbers for the whole class have been totaled up, record these totals on your work sheet.

8. Complete the Data Analysis work sheet.

Name ______________________________

Date ____________________ Hour __________

ALBINO PLANT WORK SHEET

Data from your own pair of groups:

Number of plants on "light" plate		
Color	Observation #1 Light incubated	Observation #2 Light incubated
Green		
White		

Number of plants on "dark" plate		
Color	Observation #1 Dark incubated	Observation #2 Light incubated
Green		
White		

Data from the whole class:

Number of plants on "light" plates				
	Observation #1 Light incubated		Observation #2 Light incubated	
Group	Green	White	Green	White
Totals				

Number of plants on "dark" plates				
	Observation #1 Dark incubated		Observation #2 Light incubated	
Group	Green	White	Green	White
Totals				

C.1

Name ______________________________

Date ____________________ Hour __________

DATA ANALYSIS

Use the data collected by the class as a whole to answer the following questions.

1. What ratio of green seedlings to white seedlings was present in the light-incubated seedlings on the first day of observation?

   First express this as a ratio of the total numbers of green seedlings and white seedlings observed. (For example, 461:119)

   ____

   Now divide the larger by the smaller number to express it as an exact ratio. (For example, 461:119 = 3.87:1)

   ____

   Now convert this exact ratio to the nearest integral ratio. (For example, 3.87:1 ≈ 4:1)

   ____

2. What did you expect the ratio of green seedlings to white seedlings to be, given that this difference in color has a simple genetic basis? Explain.

   ____

   ____

   ____

3. Do you think that the difference between the exact ratio that you calculated for Question 1 and the expected ratio that you gave for Question 2 is significant? Explain.

   ____

   ____

   ____

C.1

Name ______________________

Date ______________ Hour __________

4. What ratio of green plants to white plants was present in the dark-incubated seedlings on the first day of observation?

5. Is this significantly different than the ratio of green plants to white plants that was present in the light-incubated seedlings on the first day of observation?

6. Formulate a hypothesis to explain this difference between the light-incubated and dark-incubated seedlings.

7. How could your hypothesis be tested?

8. Compare the ratios of green seedlings to white seedlings that were observed in the dark-incubated seedlings on the first observation and the second observation.

   First observation: ______________________

   Second observation: ______________________

9. How do you account for any difference between these two ratios?

10. What overall conclusion can you draw from the data collected in this experiment?

C.1

Name ______________________

Date ______________ Hour ________

AN OPTIONAL STEP: A CHI-SQUARE ANALYSIS OF THE ALBINO PLANT DATA

1. Following the instructions for How to Perform a Chi-Square Test on Any Data Set (see Chapter 2, Section E.3) and using the data collected by the class as a whole, calculate χ^2 and p for the light-incubated seedlings on the first day of observation.

   χ^2:________ p:________

2. Based on the value of p that you obtained, do you think that the class data for the light-incubated seedlings are consistent with the hypothesis that seedling color is determined by a pair of alleles that exhibit a simple dominant-recessive relationship? Explain.

3. Repeat the calculation of χ^2 and p for the light-incubated seedlings on the first day of observation, using only the data collected by your own group.

   χ^2:________ p:________

4. Based on the value of p that you obtained this time, do you think that your own data are consistent with the hypothesis that seedling color is determined by a pair of alleles that exhibit a simple dominant-recessive relationship? Explain.

5. Which data set gave you the higher p value, the class data or your own data? Explain.

6. Do you think you need to perform a χ^2 test to determine whether the data that your class collected with respect to the dark-incubated plants during the first observation are consistent with the proposed hypothesis? Explain.

C.2

Heart Disease: A Personal Gene-Environment Interaction

INTRODUCTION

THE IDEA THAT GENES and environment interact to determine phenotype is not just an abstract concept that applies only to test organisms in laboratory experiments. It is an inescapable reality that affects each of us throughout our lives. Moreover, the environment with which our genes must interact to influence our health is largely the environment that we generate by our own lifestyle choices.

Nothing illustrates these principles more clearly than human heart disease. Heart disease remains the principal cause of death in the United States. It is a clear example of what is called a **multifactorial disease,** a disease that is caused not by any one factor but by the interplay of several factors, including genes and lifestyle. Although heart disease is being used as the example in this exercise, it is not an isolated example; most serious human diseases are multifactorial. Before going on, we should point out one common misconception about the environmental risk factors involved in heart disease: namely, that they only matter when you get older. This leads to the attitude that "It's OK to eat greasy junk food, smoke and drink while you're young, because you can always clean up your act later on when it matters." It is true that one's health is likely to be improved by "cleaning up one's act" at any age. Nevertheless, it is now very clear that the clogging of the arteries that eventually leads to heart disease can begin in early childhood as a result of poor diet and lack of exercise.

The genetic factors contributing to heart disease are many and complex. There is no single heart disease gene that will cause heart disease if you have one allele and prevent heart disease if you have a different allele. Instead, there appear to be many genetic loci at which particular alleles increase and other alleles decrease the probability that you will get heart disease.

Fortunately or unfortunately, there presently is no way to determine which alleles at most of these loci a particular individual possesses. But some clues come from family history. If many members of your family have had heart disease in the past, it is fairly likely that you have inherited one or more alleles that increase your **heritable predisposition** (genetic risk) of developing heart disease. However, a complete absence of heart disease from your family history provides no assurance that you are free of genetic risk factors.

C.2

Whatever the level of one's genetic risk for heart disease, lifestyle choices play a terribly important role in determining whether or not heart disease will actually strike, and if so, at how young an age and how severely. Among the most important environmental risk factors are diet, exercise, smoking, alcohol or drug abuse, high blood pressure, and stress. The way in which these factors interact with various genetic factors is quite complex and poorly understood. But one thing is certain: The more genetic and environmental risk factors that you possess, the more likely it is that you will eventually develop heart disease.

In this exercise, we will run a simulation that should permit you to understand how genetic and environmental risk factors interact to determine the probability that one will experience a multifactorial disease like heart disease. For practical reasons, the assumptions that have gone into setting up this simulation have been simplified greatly relative to conditions in the real world. For example, only three genes will be considered in the simulation, and it will be assumed that the various genetic and environmental risk factors are all strictly additive in their effects. In reality, it appears that many genes are involved, and that various genetic and environmental risk factors may interact in a multiplicative, rather than an additive, manner.

MATERIALS

For each group of four or five students:

- 4 labeled envelopes containing Environmental Risk Cards
- 2 containers each containing 12 poker chips or other objects in three colors

C.2

Name ____________________

Date ____________ Hour ________

HEART DISEASE WORK SHEET

1. Determine your environmental (lifestyle) risk. Draw one slip (without looking) from each of the envelopes (A, B, C, and D) that your teacher will pass around. Record the information on that slip in the table below. Add the numbers to get your total environmental risk score.

Envelope	Characteristic	Personal attribute	Score
A	Weight		
B	Diet		
C	Smoking		
D	Exercise		

My total environmental (lifestyle) risk = ____________

2. Determine your genetic risk. Two cups containing poker chips or other objects of different colors will now be passed around. The different colored chips will represent alleles with different risk values. One cup will be labeled "Mother's Genes," and the other will be labeled "Father's genes." When one of these cups reaches you, close your eyes, stir the chips, and pick three chips with your eyes still closed. Open your eyes, record the colors of the chips in the spaces below, and then return the chips to the cup and pass the cup on to the next person. After all students have selected and recorded the colors of their three chips, your teacher will announce the risk score associated with each color of allele. Record these scores below, add up your genetic risk, and then add up your total heart disease risk.

Colors of "Mother's Genes:" 1. __________ 2. __________ 3. __________

Points for "Mother's Genes:" 1. __________ 2. __________ 3. __________

Colors of "Father's Genes:" 1. __________ 2. __________ 3. __________

Points for "Father's Genes: 1. __________ 2. __________ 3. __________

My total genetic risk = ____________

3. Determine your total risk.

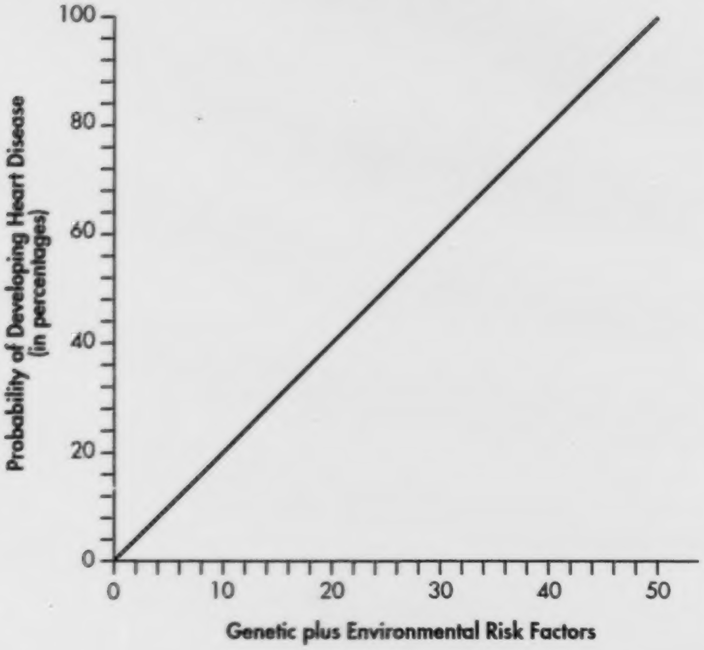
My total risk of contracting heart disease = lifestyle risk + genetic risk = ____________

C.2

Name ____________________________________

Date ____________________ Hour __________

4. Using the graph below, determine your probability of developing heart disease.



5. If this were my actual risk score (as opposed to my simulated risk score),

my probability of contracting heart disease would be about _______%.

6. My calculated risk of getting heart disease is _______% due to my environmental

(lifestyle) factors and _______% due to genetic factors.

7. Indicate the most important changes you could make in your lifestyle changes to lower your risk of heart disease if your simulated risk scores were your actual risk scores.

____

____

____

8. By doing the above, I could lower my probability of developing heart disease to about

_____%.

CHAPTER 3

How Genes and the Environment Influence Our Health

SECTION D

What Are Some of the Features of "Simple" Genetic Diseases?

D.1

Some "Simple" Heritable Defects

INTRODUCTION

GENES AND THE PROTEINS that they encode are the source of our life and health. Our well-being depends on the proper functioning of thousands of proteins, each of which is encoded by its own gene.

Occasionally, however, one of these genes undergoes a mutation that seriously impairs the function of the corresponding protein. In any individual unfortunate enough to inherit two copies of the mutant gene, any cells that are dependent on the proper functioning of the corresponding protein are in danger. That, in turn, places the whole individual in danger.

More than 4,000 human diseases are now known in which the disease symptoms are primarily a consequence of functional abnormalities of a single mutant protein. The table in Section D.1 lists just a few of the more well-known diseases of this type. Fortunately, even the most common of these diseases are quite rare.

Diseases of the sort listed in the table are commonly called **single-gene diseases** to distinguish them from a disease like heart disease, which (as we saw in the last exercise) has a multigene, multifactorial basis. However, it is now realized that such a distinction is not as meaningful as it might first appear to be. In Section D.2, PKU, one of the most well-known of the single-gene diseases, will be used to illustrate this.

D.1

A FEW HUMAN CONDITIONS CAUSED PRIMARILY BY A HERITABLE DEFECT IN A SINGLE PROTEIN

Condition	Protein Affected
Albinism (lack of pigment in skin, hair and eyes)	tyrosinase, an enzyme required to produce melanin from the amino acid tyrosine
Bubble-baby syndrome (failure to make antibodies; the heritable equivalent of AIDS)	adenosine deaminase, an important blood-cell enzyme
Cretinism (severe deficiencies of physical and mental development)	an enzyme required to make the hormone thyroxin from tyrosine
Cystic fibrosis (thick mucus in lungs and many other abnormalities, leading to early death)	a membrane protein required for normal ion balance and secretory activity of cells
Emphysema (loss of elasticity of air sacs in lung)	α1-antitrypsin, an inhibitor of the protein-digesting enzyme, trypsin (symptoms are aggravated by smoking)
Gout (arthritis-like inflammation of joints)	hyperactivity of an enzyme required for metabolism of ribonucleotides (symptoms strongly affected by diet)
Hemolytic anemia (breakdown of red blood cells)	any one of at least 14 different red blood cell enzymes
Hemophilia (severe hemorrhaging from minor injuries)	any one of 12 different enzymes required for normal blood clotting
Huntingtons disease (progressive mental and physical deterioration, usually beginning after age 40)	a brain protein called *huntingtin*, the normal function of which is uncertain and the mutant form of which accumulates in the nuclei of brain cells, leading to progressive loss of brain functions
PKU, or phenylketonuria (severe brain damage unless dietary intake of the amino acid phenylalanine is restricted)	phenylalanine hydroxylase, an enzyme required to dispose of excess dietary phenylalanine
Sickle-cell anemia (red blood cells deform and block capillaries, causing severe pain and tissue damage)	the β-globin part of the hemoglobin molecule (symptoms intensified by vigorous exercise and high altitudes)
Tay-Sachs disease (blindness, seizures, mental deterioration, early death)	hexosaminidase, an enzyme required for the breakdown of certain sugar-lipid compounds in brain cells
Xeroderma pigmentosum (mottled, thick, scaly skin condition, usually terminating with fatal skin cancer)	a DNA endonuclease required for repairing DNA that has been damaged by ultraviolet light and has not been cured via photo repair

D.2

Phenylketonuria (PKU) Illustrates the Complexities of Some "Simple" Genetic Diseases

PHENYLALANINE IS ONE OF the 20 amino acids present in nearly every protein molecule of every living creature. Humans cannot synthesize the phenylalanine that they need to make their proteins, so none of us could survive without phenylalanine in our diet. However, since phenylalanine is present in nearly every protein molecule in every kind of food we eat, most of us get more phenylalanine from our food than we actually need. Aspartame, the artificial sweetener that is also known as *NutraSweet* and used to sweeten many diet foods, now serves as important source of phenylalanine in some diets, because aspartame is about 50% phenyalanine. Getting extra phenylalanine is no problem for most of us, because we have an enzyme called phenylalanine hydroxylase that converts any extra phenylalanine into tyrosine, another amino acid that we need for making proteins.

However, some people have a mutant gene that encodes a nonfunctional phenylalanine hydroxylase. As a result they are unable to convert phenylalanine to tyrosine. Instead, their body converts any extra phenylalanine that they eat into toxic substances called **phenyl ketones,** which eventually show up in their urine. Such people are said to have **phenylketonuria,** or **PKU.** Phenyl ketones can cause serious brain damage, especially in babies. Therefore, unless PKU is diagnosed and treated at birth, children with it can quickly develop brain abnormalities and very severe mental handicaps. In most developed countries, however, all babies are tested for PKU immediately after birth, and those with it are immediately placed on a low-phenylalanine diet that includes artificial milk. Such a diet permits normal brain development.

PKU seems like a clear-cut example of a "simple" single-gene disease. It occurs only in people who are homozygous for the mutant allele that encodes a defective phenylalanine hydroxylase enzyme. Heterozygous individuals are symptom-free. However, PKU is also a clear example of the concept implied by the title of this section: namely, that even single-gene diseases can be surprisingly complex.

Although PKU is always thought of as a genetic disease, it clearly illustrates the principle that all aspects of the phenotype are a result of a gene-environment interaction. The symptoms of PKU can be almost completely prevented by simply modifying the environment – specifically, by removing phenylalanine-rich foods from the diet from early childhood on.

D.2

Another complication is that PKU is actually a set of closely related diseases, rather than a single disease. More than half of all individuals with PKU are homozygous for the most common mutant allele of the gene encoding phenylalanine hydroxylase. However, most of the remaining PKU individuals have one copy of that most-common mutation in combination with one of many other less-common mutant alleles. These different less-common mutations cause different degrees of phenylalanine hydroxylase deficiency, and thus result in different degrees of mental retardation in untreated children.

PKU is a single-gene disease in the sense that it only occurs in individuals who have two mutant alleles of the gene encoding phenylalanine hydroxylase. However, in another sense it is a multigene disease. This is because many other genes determine how severe the symptoms of PKU will be in untreated babies. Children who are from different families but have the same mutant alleles and similar diets, can have very different degrees of mental retardation. And a few such children are nearly symptom free. The nature of these other interacting genes is largely unknown, but it is thought that some of them probably control factors regulating how sensitive the cells in the developing brain are to phenyl ketones present in the blood.

In short, it is appropriate to consider the mutant genes encoding phenylalanine hydroxylase as the principal cause, but not the sole cause, of PKU. The disease is clearly multifactorial.

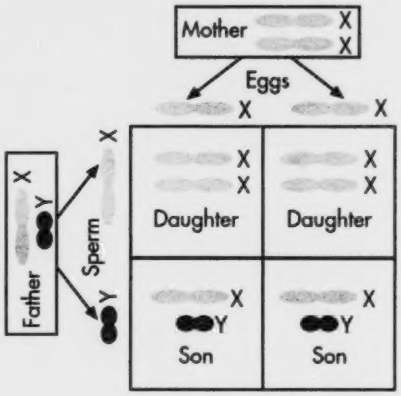
A further complication of PKU has been discovered recently. Women who are homozygous for the mutant PKU allele but have been on a low-phenylalanine diet all of their lives can be free of any PKU symptoms. But sometimes these women have babies who are born with PKU-like brain defects. Such brain defects occur even in babies who are not homozygous for the mutant PKU allele themselves. Apparently a low-phenylalanine diet is adequate to protect the mother – but sometimes not her unborn baby – from the adverse effects of PKU.

It is known that many other so-called single-gene diseases exhibit similar complexities. Probably most of them do. However, hundreds of diseases in this category are so rare that they have never been studied in any detail.

D.3

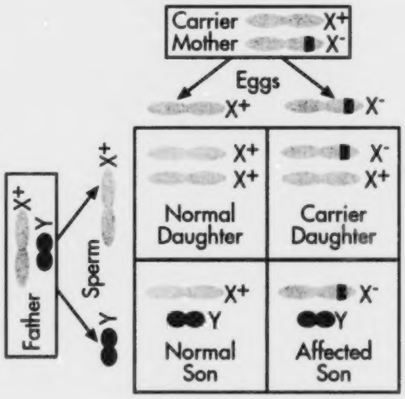
The Special Inheritance Patterns of Sex-Linked Mutations

IN HUMANS AND ALL other mammals, gender is determined by the X and Y chromosomes, which are known as the **sex chromosomes.** Whereas females possess two X chromosomes, males possess one X and one Y chromosome. This results in very specific chromosome inheritance patterns. Children of both sexes inherit an X chromosome from their mother. But whereas girls also inherit an X chromosome from their father, boys never do. They always inherit a Y chromosome from their father, as shown in the Punnett square to the right.



Most genetic disorders occur in males and females with about the same frequency. But certain disorders occur much more frequently in males than in females, and most frequently in men whose mother's father had the same condition. Some examples are red-green color blindness, hemophilia, and Duchenne muscular dystrophy.

These conditions are known as **sex-linked disorders,** because they are a result of mutations of genes that are located on the X chromosome. Thus, they show an inheritance pattern just like the inheritance pattern for the X chromosomes. If a boy develops a sex-linked disorder such as hemophilia (in which blood fails to clot normally), there is a very high probability that his mother is a **carrier** for the disease. That is, that she is heterozygous for the hemophilia mutation, which is recessive. (In rare cases, the boy's hemophilia could be due to a new mutation that occurred in the nucleus of the egg from which he was derived.) We can see how such diseases are transmitted in the Punnett square to the right, in which the mother's normal chromosome is labeled X+ and the one carrying the mutant allele is labeled X-. The father's X chromosome is also labeled X+.



D.3

Name ______________________________

Date ____________________ Hour __________

SEX-LINKED MUTATION WORK SHEET

1. If a woman is a carrier for a mutation causing a sex-linked disorder, what is the chance that one of her sons will have the disorder? Explain.

2. If a woman who is a carrier for a sex-linked disorder already has one son who has the disorder, what is the chance that if she has a second son he will also have the disorder? Explain.

3. If a man has a sex-linked disorder, what is the chance that he will pass it on to one of his sons? Explain.

4. If a man has a sex-linked disorder, what is the chance that one of his daughters will be a carrier for that disorder? Explain.

5. If a man has a sex-linked disorder, what are the chances that one of his grandsons will inherit that disorder? Explain.

6. It has been postulated that a condition known as "hairy ears" is caused by a mutation of a gene on the Y chromosome. Assuming that this is true, what is the chance that one of the sons of a man with hairy ears will inherit the "hairy-ear mutation?" Explain.

7. What is the chance that one of the daughters of the man referred to above will pass the "hairy-ear mutation" on to one of her sons? Explain.

D.4

Investigating Human Genetic Diseases

INTRODUCTION

HUMAN DNA CONTAINS ABOUT six billion base pairs packed into 46 chromosomes in each of the approximately four trillion cells in a human body. So it's not too surprising that there are many different things that can go wrong with human genes. Indeed, the most surprising fact may be that we do not all suffer from some serious genetic defect.

The only feature shared by all genetic diseases is that they involve some kind of mutation affecting one or more genes. However, there are several different kinds of mutations that can be present, and they differ with respect to their origins and their heritability patterns.

One important distinction is between **germline mutations,** which are mutations that are passed on in eggs and/or sperm and thus end up in every cell of the offspring, and **somatic mutations,** which originate within a single body cell, and thus are present in only that cell and its direct descendants. Germline mutations can be passed on from generation to generation and are the basis for most heritable diseases such as sickle-cell anemia and PKU. Somatic mutations cannot be passed on to one's offspring, but can cause serious diseases, such as cancer. Although a cancer can damage essential organs, and therefore the entire body, it always starts out as a genetic change within a single cell of the body, most often a change in one of the genes that controls cell division.

Another important distinction is between **point mutations,** which involve DNA sequence changes at one point within a single gene, and **chromosomal mutations,** which involve abnormalities affecting large chromosomal regions or even whole chromosomes. In most cases the diseases listed in Table D.1 and many others are the result of point mutations. But some serious diseases, for example Down syndrome, are the result of having one extra chromosome. Such a problem indicates that the chromosomes did not separate properly at the meiotic division that was involved in formation of either the sperm or the egg.

Other distinctions involve differences of the sort that you have already encountered – such as the distinction between dominant and recessive mutations and between single-gene and multigene diseases.

A final distinction to be made is between sex-linked mutations, which are mutations of genes that are located on the X (or occasionally the Y) chromosome, and **autosomal mutations,** which are mutations of genes that are located on any other chromosome.

D.4

PRODUCING A PAMPHLET FOR A GENETIC COUNSELOR

In this exercise, you are to produce a pamphlet about some particular genetic disorder. The intent is to produce a pamphlet of the sort that could be displayed in a genetic counselor's waiting room for parents and family members to pick up and read. You should supply information regarding the following topics:

1. Title of the disorder. (You may want to create an interesting title for your brochure, but avoid being too cute. Remember that for an affected family seeking help, a genetic disease can be very serious business.)

2. Symptoms of the condition. (Find as many as you can, list them, and describe them clearly and concisely.)

3. The type of genetic change that causes this condition (select one).

   a. A point mutation involving a base substitution, deletion, or addition; autosomal or sex-linked; dominant or recessive. Explain how this kind of mutation occurs and is transmitted from generation to generation.

   b. A chromosomal mutation involving a deletion, duplication, translocation, or inversion of a piece of a chromosome. Explain how this kind of chromosomal mutation occurs.

   c. A missing or extra chromosome. Explain how this kind of chromosomal abnormality occurs.

4. The frequency with which this condition occurs in the population. Does it occur more frequently in certain human populations than in others?

5. Environmental factors (such as diet, exercise, exposure to sunlight) that may be known (or even thought) to affect the severity of the disease.

6. Treatments, if any, for this condition.

7. Facilities that offer treatments for individuals suffering from this condition and/or emotional support for members of the family. (Hospitals, genetic counselors, support groups, websites, Yellow Pages) Be specific.

To investigate these questions, you may use library reference books, medical and general encyclopedias, genetics textbooks, and the Internet. Websites have been created to provide information about many such genetic disorders.

The topics listed above should be the basis for sections of your pamphlet. As you are writing, remember that your audience is made up of individuals whose understanding of genetic terms and concepts may be quite limited. At the end of your pamphlet, list two references in acceptable bibliographic style. You may use clip art or your own artwork to enhance your pamphlet.

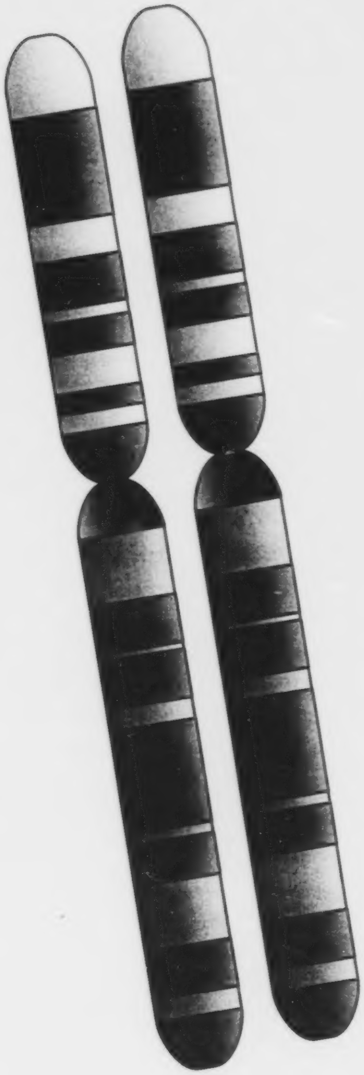
The grading of your pamphlet will be based on:

1. Evidence that you accessed, analyzed, and synthesized the relevant information.
2. The completeness and accuracy of the information that you presented.
3. The clarity with which you expressed this information.
4. The overall appearance of your finished pamphlet.

Below is a sampling of genetic diseases that you might investigate and produce a pamphlet about.

achondroplasia
breast cancer
club foot
color blindness
coronary artery disease
cystic fibrosis
Down syndrome
fragile X syndrome
Huntingtons disease
Klinefelter syndrome
Marfan syndrome
phenylketonuria
Prader-Willi syndrome
retinoblastoma
spina bifida
trisomy 13 syndrome
Wolf-Hirschhorn syndrome
beta-thalassemia
cleft palate
colon cancer
congenital hip dysplasia
cri-du-chat syndrome
diabetes, non-insulin dependent
Duchenne muscular dystrophy
hemophilia
hypertension
Lesch-Nyhan syndrome
neurofibromatosis
polycystic kidney disease
pyloric stenosis
sickle-cell anemia
Tay-Sachs disease
Turner syndrome

There are many other possibilities. If you have come across a disease in your reading that interests you as a possible research topic, but is not included in the above list, discuss it with your teacher.



CHAPTER 3

How Genes and the Environment Influence Our Health

SECTION E

How Does a Genetic Counselor Detect Mutant Genes?

E.1

Detecting the Duchenne Muscular Dystrophy (DMD) Mutation

IN THIS EXERCISE WE will pretend that you are a technician in a genetic counselor's laboratory. You will perform a DNA screening test that is similar to the one used by real genetic counselors, and then you will interpret the results and decide how to present this genetic information to the family that is involved.

The situation you will be dealing with is the following: Mary and John Smith have three children: Daniel, age 5; Alice, age 4; and Michael, age 1. Mary is two months pregnant. Recently Mary and John noticed that Daniel was having trouble climbing the stairs. He also complained several times that he was really tired after playing tag with his sister. Daniel's doctor suggested some medical tests, which brought the family some bad news: Daniel has a disease called Duchenne muscular dystrophy (DMD).

Mary and John had never heard of DMD before, so they asked the doctor a lot of questions and went to the library for more information. They learned that DMD is a sex-linked genetic disease, which means that it results from damage to a gene on the X chromosome. That is why almost everyone with DMD is male. A girl may inherit an X chromosome with a defective copy of the DMD gene from her mother and like her mother, she will be a carrier for DMD. But the girl most likely will be protected from developing DMD by the normal X chromosome she gets from her father. In contrast, a boy does not get an X chromosome from his father, and if the X chromosome he gets from his mother carries a defective copy of the DMD gene, he will develop DMD.

Usually boys with DMD are healthy until the age of 4 or 5, at which time their muscles start to weaken. The doctor told the Smiths that Daniel would probably need a wheelchair in a few years, and that he would probably die before the age of 21. Although scientists are working to find a cure for this disease, there is no effective treatment for DMD now.

Obviously, Mary and John were very upset by this news about Daniel. Then they began to worry about their other children. Because Mary was a carrier for DMD, it was possible that Michael would develop DMD also, although he was too young to show any symptoms yet. They also worried about their unborn child and whether he or she might be at risk for DMD. Finally, even though Alice would not develop DMD, the Smiths wanted to find out whether she was a carrier like her mother. When the doctor explained that there is a genetic test that could determine whether each family member had the defective gene for DMD, Mary and John decided that they wanted this information. So the doctor sent them to a genetic counseling center where the tests could be done.

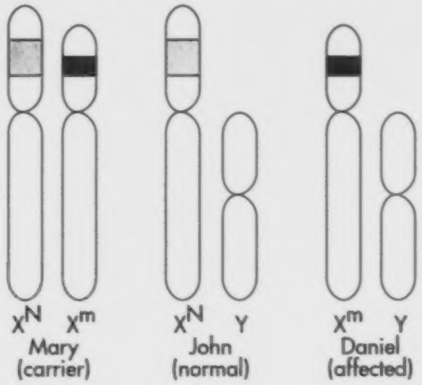
E.1

First the genetic counselor met with Mary and John and reviewed with them all of the information about DMD that they had received earlier. She tried to make sure that they understood the nature of the test that was to be performed, what kinds of information it could provide them, and how they might choose to use that information once they had it. When the Smiths assured their counselor that they understood, and that they did want to have the test performed, you, as lab technician, took a small drop of blood from a finger of each family member. Mary also went through a process of fetal blood sampling, so that the DNA from her unborn child could also be tested. The genetic counselor told Mary and John to come back in a week to find out the test results.

You took the Smith's blood samples and isolated DNA from the white blood cells in each of them. But the DMD gene that the Smith family wished you to analyze was only one of many thousand genes in each of the resulting DNA samples. So, to get enough of this particular gene to study, you used a procedure called **PCR** (short for **polymerase chain reaction**) to amplify (make many copies of) the tiny section of DNA that contained the DMD gene.

PCR uses **DNA polymerase** (the same enzyme that cells use to replicate their DNA before they divide) to replicate, over and over again, a particular DNA region of interest. A single round of replication takes only about two minutes. Then both the copies made in that first round can be replicated again, and so on. At this rate of doubling, PCR can produce over a billion copies of a piece of DNA in about an hour. With so many copies of that gene in each tube, it becomes much easier to compare genes from different individuals and see if they are the same or different

You have used PCR to make billions of copies of the DMD genes from the DNA sample taken from each member of the Smith family. Now your next job is to analyze the samples and determine which ones contain only copies of the wild-type DMD allele, which ones contain only copies of the mutant DMD allele, and which ones contain copies of both DMD alleles. As an expert, you know that most mutations that cause this disease involve a deletion of part of the DNA from the DMD gene, so that the mutant alleles will be shorter than the wild-type alleles. As illustrated in the diagram, this implies that Mary, who is a carrier for DMD, has one normal and one short (mutant) allele at the DMD locus. Since John, being a male, has only one X chromosome and does not have DMD, his DMD allele must be of normal length. Daniel has the DMD disorder, so he must have received the X chromosome with the defective DMD gene from his mother.



Now the question is, Which DMD alleles do the other family members have? That's what you will try to determine.

E.1

DNA molecules that differ in length can be separated and analyzed by a process known as **agarose gel electrophoresis.** This method uses an electric current to push DNA molecules through a gel-like substance called **agarose.** Small DNA molecules move through the gel faster than large ones.

You will use agarose gel electrophoresis to simulate the procedure that the genetic counseling lab would use to determine the genotypes of a set of family members with respect to a gene of interest – such as the DMD gene. In a real diagnostic test, PCR-amplified DNA samples derived from the blood cells of the various members would be subjected to gel electrophoresis, and then the gel would be stained to make the DNA fragments visible. Each family member's DNA sample would then be seen to contain one or both of two different sizes of DNA fragments: small or large. From this information, the counselor would then determine whether each individual had only the normal allele, only the mutant allele, or one of each (and therefore was a carrier).

Because we do not have a real Smith family, a real PCR machine, or a real PCR technician available in our classroom, we will use two dyes of slightly different color to represent the mutant and normal DNA fragments of interest. Each sample you receive for electrophoresis will contain one or both of these dyes. The dyes are ones that (like DNA molecules of different lengths) move through a gel at different rates when subjected to an electric field. The faster-moving dye will represent the mutant DMD allele, and the slower moving one will represent the wild-type allele. Your job will be to determine which allele(s) each member of the Smith family possesses, by inspecting the gel at the end of the electrophoresis.

E.1

MATERIALS

For each group of students (group size to be determined by equipment availability):

- 1 precast agarose gel, or 1 gel-casting tray plus masking tape, 1 or 2 gel-casting combs and 50 ml of 0.8% agarose in water
- a gel electrophoresis chamber and power supply
- a small container of tap water
- 1 20 µl micropipettor
- a weighing boat or piece of white paper
- 6 dye samples labeled A-F
- 6 pipette tips

PROCEDURE

If your teacher provides you with a pre-poured agarose gel, you will begin at step e. below.

1. To prepare the agarose gel:

   a. Seal the ends of the gel-casting tray with masking tape. Insert the comb near one end of the casting tray. If your teacher instructs you to put two combs in your tray, so that two groups of students can run their samples at the same time, the second comb should be placed just beyond the center of the tray. Place the tray on a level surface where it will not be disturbed while the gel solidifies.

   b. Carefully obtain a flask with melted agarose solution from the water bath or hot plate.

   c. Carefully pour about 50 ml of the agarose solution into the casting tray, to fill it to a depth of about 4 mm. The gel should cover only about one-third of the height of the comb teeth.

   d. Do not move or jar the casting tray while the agarose solidifies. As it becomes solid (10-15 minutes), the agarose will change from clear to cloudy.

   e. When the agarose is solid, carefully remove the tape from the casting tray to expose the ends of the gel.

   f. Take the gel to the area where the gel electrophoresis chamber has been set up next to the power supply. Place the gel in the electrophoresis chamber so that the end containing the comb is toward the black electrode. Fill the chamber with tap water to a level that just covers the entire surface of the gel.

   g. Slowly and gently remove the comb, taking care not to rip the gel. The water will lubricate the comb so it pulls out more easily.

   h. Make certain that the wells (holes) left in the gel by the comb are completely covered with a layer of water. If you notice "dimples" around the wells, slowly add water until they disappear.

2. To load the samples:

   a. Use a micropipettor to load 5 µl of each sample into separate wells as shown in the diagram below. Leave one well empty at each end (fig. 1).

   b. Using two hands, steady the micropipettor over a well.

   c. Carefully dip the pipette tip through the surface of the water, center it over the well, and then gently depress the pipette plunger to slowly expel the sample into the appropriate well. If the tip is centered over the well, the DNA will sink to the bottom of the well. Do not release the plunger until the tip is out of the buffer.

   d. As one person adds the samples to the wells, another member of the group should label the diagram on the DMD Diagnosis Work Sheet to indicate which sample went in which well.

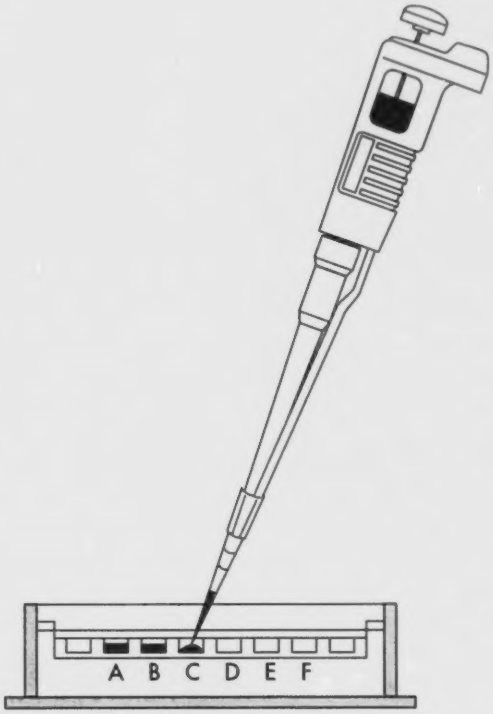


Figure 1

   e. If two groups of students are to run their samples in the same gel, one group of students should finish putting their samples in one set of wells before the second group begins to add their samples to the other set of wells.

E.1

3. To separate DNA through electrophoresis:

   a. Avoid moving or bumping the electrophoresis chamber as you do the following: Close the top of the electrophoresis chamber and connect the electrical leads to the power supply, red to red and black to black. Make sure both electrodes are connected to one channel of the power supply.

   b. Notify your teacher that you are ready to run your gel.

   c. As soon as you get your teacher's approval, set the power source to 130 volts and turn on the unit.

   d. The dial should now register approximately 50-100 milliamps. This confirms that current is flowing through the gel. If you do not detect a current, turn off the power supply, check the connections, and try again.

CAUTION: Electric shock hazard! Do not put fingers or other objects into the box while power supply is on.

   e. Shortly after the current is applied, you should see the samples moving into the gel.

   f. Run the electrophoresis for approximately 15 minutes. Check to monitor the progress of the dye bands. (If you leave the gel running for too long, the dyes will run out the end of the gel and get lost in the water.)

   g. When the dyes have moved far enough to be clearly distinguishable, turn off the power supply. Then disconnect the leads.

   h. Carefully remove the gel from the electrophoresis chamber and place it in a weighing boat or on a piece of white paper for viewing. Then complete the DMD Diagnosis Work Sheet.

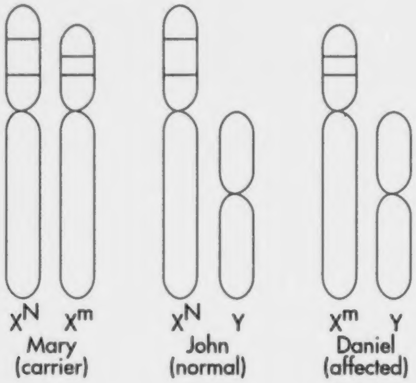
E.1

Name ____________________

Date ____________ Hour ________

DMD DIAGNOSIS WORK SHEET

1. On the diagram below, color the defective alleles purple and the normal alleles blue. NOTE: Deletion not to scale.


X^N
X^m
Mary
(carrier)
X^N
Y
John
(normal)
X^m
Y
Daniel
(affected)

2. On the diagram below, a) above each well, put the letter that was on the sample that was loaded into that well and b) using colored pencils, draw all the bands that you observed on your gel after electrophoresis.

E.1

Name ______________________________

Date ____________________ Hour __________

3. Fill in each of the blanks in the data table below:

Sample	Family Member	# of DMD Alleles	Genotype	Status
Tube A	Mother Mary	2	$X^N X^m$	Carrier
Tube B	Father John	1	_____	Healthy
Tube C	Son Daniel	1	_____	Has DMD
Tube D	Daughter Alice	_____	_____	_____
Tube E	Son Michael	_____	_____	_____
Tube F	Fetus M or F (circle)	_____	_____	_____

4. Which allele moves further into the gel, the normal (X^N) or mutant (X^m) allele? Why?

____

5. Is the daughter, Alice, a carrier for DMD? How can you tell? ____________________

____

6. Does Michael have DMD? How can you tell? ____________________________

____

7. What can you tell the Smith family about their unborn child? ____________________

____

8. Why are most patients with DMD male? ______________________________

____

9. Can a boy be a carrier for DMD without having the disease? Why or why not?

____

10. If you were the genetic counselor in this case, what would you tell the Smiths about their test results?

____

Career

CHAPTER 3

How Genes and the Environment Influence Our Health

SECTION F

How Can I Become a Genetic Counselor ?

Career **F.1**

How Can I Become a Genetic Counselor?

A GENETIC COUNSELOR WORKS with individuals or families who are afflicted with a genetic disease or are concerned that they might be. As knowledge has increased, the list of genetic diseases has been extended to include such complex, multifactorial conditions as diabetes, heart disease, and Alzheimer's, in addition to the more classical single-gene diseases (PKU) and chromosomal diseases (Down syndrome).

In many cases families with a sick child will be referred to a genetic counselor by a family physician or pediatrician who suspects that the child's symptoms may be due to a particular disease that has a known genetic basis. In other cases a couple will seek the help of a genetic counselor much earlier, during early pregnancy, if their family histories suggest that their developing fetus may have some serious heritable disease. In yet other cases a couple may visit a genetic counselor even earlier, to determine whether they are carriers for a disease that runs in one or both of their families and to get advice about whether they should avoid pregnancy. But because the number and types of genetic abnormalities that can be detected by specific DNA tests is now increasing very rapidly (along with people's familiarity with genetics), a growing number of people seek the services of genetic counselors for more reasons every year.

The responsibilities of a genetic counselor may vary, but often include one or more of the following:

- helping to diagnose a disease suspected of being genetic by prescribing or performing appropriate DNA tests;
- providing the afflicted individual and/or other family members with information about the nature of the disease, its genetic basis, and the way that symptoms of the disease can be expected to change over time;
- working with the affected individual, other family members, and physicians to provide up-to-date information about various treatment options;
- informing relatives of the afflicted individual about their risks of being carriers for the disease, of being affected by it, and of passing it on to their children or grandchildren;
- prescribing or performing diagnostic tests designed to provide other family members with information that they request, regarding their own genetic status;
- providing a list of all possible options to a woman who is carrying a fetus that has been found to have a genetic liability.

Career **F.1**

It is generally agreed that one of the most important requirements for effective and ethical genetic counseling is being able to provide all of the relevant information to patients and family members in a value-neutral manner. That is to say, a genetic counselor should avoid trying to impose his or her own values on the decision making process of the patient or family. Rather, the job of the genetic counselor is to help those affected identify the course of action that they consider best in terms of their own values.

The first step toward a career in this area is to attend college and get a well-rounded education, with a strong background in the humanities and social sciences as well as an emphasis on chemistry and biology (and particularly genetics). Because genetic counseling often involves risk prediction and manipulation of probabilities, course work in statistics could prove very helpful. If it were possible to do a research project under the supervision of a faculty member who was interested in genetics, that could significantly enhance your preparation.

Such a college education would probably qualify you for a job as a technician in a genetic counseling laboratory, running diagnostic tests like the one that was simulated in the preceding exercise. However, in order to interact directly with patients and perform most of the responsibilities listed above, one must first become accredited as a genetic counselor by the American Board of Medical Genetics, and that requires studies beyond the undergraduate college level.

There are two principal routes one can follow after undergraduate studies to prepare for a position in genetic counseling. The first is to attend graduate school and study human genetics at either the MS or the PhD level. There are now several master of science programs in genetic counseling that lead to accreditation as a genetic counselor. Individuals who have followed this route generally provide patients and/or their families with information regarding the nature and possible progression of the disease that the patient is experiencing, assist family members with risk assessment, as well as ordering and interpreting diagnostic tests for other members of the family.

In order for you to be able to assume ultimate responsibility for genetic diagnosis, counseling, and treatment however, you would need to attend medical school and obtain an MD degree. Physicians with an interest in genetic counseling and treatment have traditionally specialized in pediatrics in the past, because most single-gene diseases and chromosomal diseases become apparent in infants. (Typically, about a third of all patients admitted to a pediatric hospital suffer such diseases.) However, this situation is changing, as genetics is increasingly being used to diagnose disease, assess patient risk, and select effective therapies for older individuals. Therefore, an increasing number of physicians are combining their interests and training in medical genetics with a specialization in internal medicine or family practice.

The three types of specialists discussed here (physicians specializing in genetic disease, accredited genetic counselors, and genetics lab technicians) will typically work together, often in conjunction with the patient's primary-care physician, on each case.

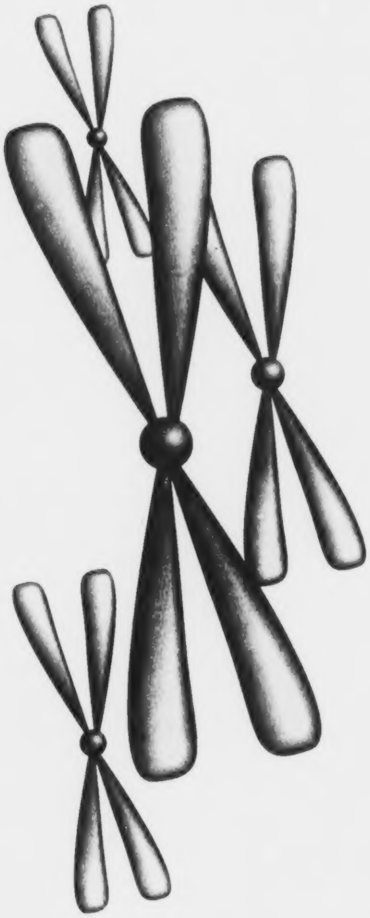
Career F.1

Additional information about preparing for such careers can be obtained from the following:

The National Society of Genetic Counselors, Executive Office
233 Canterbury Drive
Wallingford, PA 19086-6617
Telephone: (610) 872-7608
http://www.kumc.edu/gec/prof/nsgc.html

A World of Genetic Societies (links to various genetics-related sites)
http://www.faseb.org/genetics

Information about a broader range of careers in genetics is available at http://www.ornl.gov/hgmis/education/careers.html



CHAPTER 3

How Genes and the Environment Influence Our Health

CHAPTER 3

How Genes and the Environment Influence Our Health

CHAPTER THREE OVERVIEW T176

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Chapter Three Overview

TEACHING GENETICS IS A bit like walking a tightrope; to do it well, one has to exercise a fine sense of balance and avoid falling off to one side or the other. On the one hand, we want our students to understand that genes play a vital role in the living world. We need to help them appreciate the fact that every aspect of the life of every organism is influenced by its genetic endowment. On the other hand, it is equally important for them to understand that genes are not all powerful. We need to work equally hard to help them appreciate that the effect a gene will have – if any – is entirely dependent on the details of the complex environment inside and outside of the cell in which that gene resides.

While we were introducing our students to the basic aspects of molecular and Mendelian genetics in the first two chapters of this manual, our emphasis was necessarily on the importance of genes in the living world. But now it is time to begin striking the balance referred to above. In this chapter, we will introduce the students to some of the paradoxical features of genes and a few of the complexities of the gene-environment interactions that influence all aspects of our lives, including our health.

In the first hands-on exercise in this chapter, students will learn that something as apparently innocuous as the light shining on an organism can – depending on its wavelength – act either to cause or to prevent serious genetic changes, many of which could be fatal. In the second exercise they will discover unmistakable evidence that a favorable interaction between genes and environmental factors is required for the synthesis of even the most important molecule in the entire living world, the chlorophyll molecule, on which photosynthesis (and thus all of life) depends. The third exercise should not only reinforce their appreciation of the importance of such gene-environment interactions but should really bring the concept home. In this simulated risk-assessment exercise, students will discover that it is unlikely that they could have any combination of "good" genes that would protect them from a serious risk of heart disease if they make all the wrong lifestyle choices, and that it is equally unlikely that they could have any combination of "bad" genes that would doom them to suffer serious heart disease if they adopt a truly healthy lifestyle.

In the rest of the chapter, the students will consider additional aspects and kinds of heritable human diseases and will learn, for example, that the severity of even supposedly simple, single-gene diseases often is determined by complex interactions of multiple genes with one another and the environment. The goal of this chapter is to get the students to realize that evidence of a heritable aspect to an undesirable human trait or condition is not equivalent to evidence that nothing can be done about it. After all, appreciation of the importance of environmental changes in promoting human good – whatever the genetic endowment of the individuals concerned – is the basis for two of the world's most noble professions, education and medicine!

CHAPTER 3

How Genes and the Environment Influence Our Health

SECTION A

How Stable and How Powerful is DNA?

Chapter 3: Section A Background

WE ARE LIVING IN the middle of an amazing genetic revolution. Sequencing of the human genome is essentially complete as of early 2001. The first successful cases of human gene therapy were reported in the year 2000. In that same year a quarter of the corn seeds, more than half of the soybean seeds, and nearly three fourths of the cotton seeds planted in the United States contained foreign genes inserted by genetic engineers.

Under these circumstances, it is no great surprise that there is a strong tendency on the part of journalists and others to exaggerate the importance of genes and the DNA molecules of which they are made. Biologists and biology teachers who make careless word choices contribute to such exaggerated views of DNA potency. They might, for example, refer to DNA as a "self-replicating molecule," although they know that no DNA molecule can replicate itself and that the replication of a DNA molecule requires interactions among many different kinds of enzymes and other molecules within the living cell. The rhetoric is often as excessive and ill-considered at the whole-organism level, as research biologists call press conferences to announce that they have discovered the gene for breast cancer, or schizophrenia, or alcoholism, or obesity, or some other complex human trait. They do this despite the knowledge that every one of those conditions is a complex entity in which a wide variety of environmental factors, as well as a considerable number of genes, play determinative roles.

Surely we want to infuse our students with a sense of excitement as we introduce them to the modern age of genetics. But just as surely, we want to do it in a realistic, scientifically sound way. This section is a step in that direction.

A.1

DNA Paradoxes STUDENT PAGE 162

LESSON OVERVIEW

This short reading assignment is intended to prepare your students for some of the paradoxical features of DNA and complexities of genetics - particularly the genetic aspects of human diseases - that they will encounter in this chapter.

TIMELINE

It will take an average student about 5 minutes for to read this material.

CHAPTER 3

How Genes and the Environment Influence Our Health

SECTION B

How Do Heritable Changes in Genes Occur?

Chapter 3: Section B Background

THE MAJOR EMPHASIS OF this chapter is on genetic diseases that effect humans. However, a first step toward understanding genetic disorders is understanding the fact that genes can **mutate**, or change from one stable, heritable state to another stable, heritable state. The exercise in this section should provide your students with visible evidence of that fact. They will irradiate bacteria with ultraviolet (UV) light to induce mutations with a visible effect.

Because UV irradiation is about the easiest and surest way of inducing DNA damage experimentally, UV is one of the most extensively studied **mutagens.** It can damage the DNA in several ways, but the most common is by causing the formation of a pair of covalent bonds between adjacent thymine residues in a DNA strand, generating what is known as a **thymine dimer**. Thymine dimers cause kinks in the DNA strand that prevent both replication and transcription of that part of the DNA. Because they block DNA replication (and therefore prevent cells from reproducing), thymine dimers and other forms of UV damage cannot be inherited, and thus do not constitute mutations. Geneticists sometimes call such kinds of DNA damage **premutational lesions**, and because they prevent both transcription and replication of the genes in which they are present, these lesions are fatal if they go uncorrected. Not surprisingly, therefore, organisms (including ourselves) have a number of different DNA repair systems for eliminating such damage from DNA. But it is the very act of attempting to repair the UV-induced damage that sometimes converts it into a heritable mutation.

One of the more interesting DNA repair systems deals specifically with the thymine dimers that have been produced by UV. It is called **photo repair**, because it involves an enzyme that uses the energy of visible light to break the covalent bonds between adjacent thymines that were formed earlier by UV irradiation.

Photo repair is only one of many DNA repair systems present in cells. Other repair systems are required because UV causes more than one kind of DNA damage and UV is far from the only source of DNA damage in the living world. Many chemical compounds, both natural and man-made, can damage DNA if they get into cells. But it doesn't even take an external agent to damage DNA. It is now known, for example, that during an average day the DNA in each one of our cells loses about 5,000 of its adenine and guanine bases, due strictly to the thermal agitation that the DNA molecules experience at normal body temperature. Such **thermal depurination** is completely unavoidable at temperatures that are compatible with life, but it would be devastating to us (or any organism) if it were left uncorrected. And this is only one of several forms of spontaneous damage our DNA undergoes all of the time. It is of little surprise, then, that every living cell has several dozen different enzymes devoted to repairing DNA damage of one kind or another.

Most of these repair systems, including photo repair, are said to be **error-free**, because they restore the damaged DNA to its initial state. These systems are surprisingly efficient, accurately repairing more than 99.99% of the DNA damage that occurs in a nucleus. Without error-free DNA repair systems neither we nor any other organism would be alive. But these repair systems are not perfect; if any damage goes undetected or unrepaired, it still can be fatal to the cell. Therefore, organisms from bacteria to humans possess a repair system of last resort, known as the **error-prone** DNA-repair system. Error-prone repair is induced by thymine dimers or other forms of DNA damage that (for whatever reason) have not been repaired by any error-free repair system.* The error-prone enzymes usually cut both strands of the DNA to remove the damaged region and then patch the molecule back together with a new piece of double-stranded DNA that they make without regard to the sequence of DNA bases that were previously present in that region. Just as the yarn used to mend a hole in a piece of clothing often does not match the original cloth, the sequence of the piece of DNA that the error-prone system uses as a patch usually bears no resemblance to the sequence that was in that region originally. As a result, the life of the cell may have been saved by the repair job, but it now has a heritable change – a mutation. Indeed, as already mentioned, it is such error-prone repair of damaged DNA that causes a mutation, not the initial damage itself. The significance of such a mutation will depend, of course, on the nature of the gene in which it occurs and the nature of the change that it causes.

---

* The most common form of error-prone DNA repair system in organisms from bacteria to humans is called **SOS repair**. SOS refers, of course, to the internationally recognized distress call that stands for “Save Our Ship.”

B.1

Inducing Mutations with UV Light

STUDENT PAGES 164-167

LESSON OVERVIEW

In this exercise, your students will expose the bacterium *Serratia marcescens* to UV light to induce mutations with a visible effect, the loss of the ability to form the red pigment that characterizes wild-type *Serratia*.

The red pigment made by wild-type *Serratia* is called **prodigiosin**. It is a substance that is toxic to certain other microorganisms and therefore acts as an **antibiotic** that enables *Serratia* to compete more effectively with other microorganisms in its normal environment. Synthesis of prodigiosin requires the products of two separate and quite complex metabolic pathways. Because mutations that affect any of the steps in either of these pathways will interfere with pigment production, loss of pigmentation occurs with much higher frequency than a phenotype due to mutation of any single gene would.

The level of UV irradiation that your students will use in this experiment is one that (in the absence of photo repair) should induce a high level of error-prone DNA repair and therefore will lead to many mutations per cell, many of which will be lethal. Thus it will not be surprising if most of the bacteria that are grown in the dark die (particularly on the dishes that were given the longest UV exposure). Growth in the light should result in a substantial reduction in mutations and lethality because the light promotes error-free photo repair. But even when grown in the light, the bacteria should exhibit enough error-prone repair to produce some colonies that have lost the ability to synthesize prodigiosin, and are therefore colorless.

REFERENCES

Lewis, R. Human Genetic: Concepts and Applications, 130-133 Dubuque, Iowa: Wm. C. Brown Publishers, 1994.

Environmental Protection Agency's Stratospheric Ozone Hotline: (800) 296-1996. UV indexes, ozone layer, etc.

TIMELINE

Day 1

This part of the exercise requires 30 minutes to complete. Students spread the bacteria on nutrient agar dishes, expose them to UV, wrap them, and then set them aside for dark or light incubation. The rate limiting step will be getting the dishes irradiated, which will take 2-3 minutes per group.

Day 4 or 5

This part of the exercise should require about 20 minutes to complete. Students uncover their dishes, record their observations, and answer the questions on their work sheet.

MATERIALS

For each group of three or four students:

- 1 1000 µl micropipettor
- 1 sterile pipette tip
- 1 sterile microcentrifuge tube containing 500 µl of *Serratia marcescens* culture
- 4 nutrient agar petri dishes
- 1 marking pen
- 1 sterile inoculating loop
- disinfectant

For each class:

- 1 short-wavelength UV lamp* on a stand
- 1 watch with a second hand
- 1 waste receptacle containing 10% bleach solution
- aluminum foil
- plastic wrap
- 1 fluorescent or incandescent lamp

*It is important to use a short-wavelength UV lamp for this exercise. A long-wavelength UV lamp (sometimes know as a blacklight) will not work to induce mutations. Many UV lamps come with interchangeable filters or switches that permit them to be used in either wavelength range (for example, Carolina Biological cat. # BA-GEO9589). If you do not already have such a lamp, you may be able to borrow one from (or share the purchase price of one with) your school's earth science teacher.

ADVANCE PREPARATION

Generalized instructions for ordering and preparing microbiological media, agar dishes, etc. are given in Chapter 1, Section E.2 (pages T88 to T90).

1. Obtain a culture of *Serratia marcescens* from Carolina Biological (cat. # BA-15-5452) and use it to prepare a stock culture in liquid nutrient broth (NB) that will later be dispensed into individual tubes for student use.

   - Using sterile technique, measure appropriate volumes of sterile NB into one or more sterile, loosely capped culture tubes, allowing at least 0.5 ml of NB per lab group. (Or measure NB into non-sterile tubes, cap loosely and sterilize in an autoclave or pressure cooker.)
   - Transfer bacteria from the agar culture you received from Carolina to the NB tubes, using a sterile inoculating loop; twirl the loop in the NB to release the bacteria.
   - Incubate the tubes at room temperature under a light. Growth will be faster if you swirl the tubes carefully every hour or so during the day.
   - Incubate until the culture has developed a rich red color. Depending on the temperature of your room and the number of bacteria you transferred initially, this could take two to four days, but then the culture can be kept at room temperature for a couple of weeks with no adverse effects on the results.

2. Before class, dispense 500 μl of this *Serratia* culture to a sterile microcentrifuge tube for each lab group. Then prepare 4 nutrient agar dishes per lab group and prepare disinfectant spray bottles and waste containers (see Chapter 1, Section E.2).

3. Set up the UV lamp in an area away from student lab benches (see precautions below). The amount of UV irradiation that the bacteria receive will depend on how far above the benchtop you position the UV lamp. Because different UV lamps have different output intensities, there is no way to specify here what height will work best with your lamp. In the first year that you run this exercise, try placing the UV lamp 20-25 cm (8-10 in) above the bench top. Record the outcome so that you will be prepared to try a different height the following year if that seems advisable. The goal is to get four dark-incubated dishes on which the survival of the bacteria differs noticeably as a function of duration of UV irradiation. So if in the first year some of the dark-incubated dishes had so few surviving bacteria that they could not be easily distinguished, try placing the light higher the second year. If in the first year some had so many surviving bacteria that they could not be distinguished, try using the lamp at a lower position (or increasing the duration of the irradiation) the second year.

4. Set up a visible light source (such as a desk lamp) in the area where students will incubate their dishes.

HINTS AND TROUBLESHOOTING

1. Shortwave UV light can damage the eyes, so be sure students do not look into the UV lamp when it is lit. It is a good idea to tape cardboard blinders to the sides of the lamp to diminish the chances of accidental exposure of eyes. Brief exposure of hands to UV light won't be harmful, but students should not hold the dishes while they are being irradiated.

2. The lights used for the Wisconsin Fast Plants experiment could be used for incubation of the light-incubated petri dishes. But if these are not available, a desk lamp will be adequate. It is best to culture the dark-incubated (foil wrapped) dishes as close to the light-incubated dishes as possible. This will minimize the number of uncontrolled variables, such as temperature differences between light-incubated and dark-incubated dishes.

3. The growth rate of the bacteria will depend on the temperature of the room, particularly at night. So you may want to monitor growth on the dishes at daily intervals to determine when they will be ready for observation. Alternatively, you can schedule the observations for Day 4 or 5. If the contrast between the pigmented and nonpigmented colonies is not clear at that point, leave the dishes at room temperature for a couple of days longer and then reexamine them. (It will not be necessary to rewrap the dark-incubated dishes under these circumstances, because photo repair can only occur in the first few hours after UV irradiation.)

4. This lab lends itself well to follow-up activities. For example, students often observe that bacterial colonies on the dark-incubated, UV-irradiated dishes are larger than those on the control dishes or the light-incubated dishes, and then jump to the conclusion that this is because the bacteria have experienced beneficial mutations that cause increased growth rates. However, the size difference will usually be due to the fact that most of the bacteria on these dishes have been killed, allowing the survivors to produce larger colonies, because of the decreased competition for space and nutrients. You could use this situation to generate a discussion about developing and testing alternative hypotheses to explain an observed event. If time permits, the students could test the two hypotheses by a) growing a culture from one of the largest colonies and b) comparing the size of the colonies produced by the new culture and the original culture after they are plated at a series of different dilutions.

   Another possible follow-up is to compare the capacity of a variety of materials, such as petri lids, glass, and various plastic sheetings or bags to screen out mutagenic UV rays. This can be done by using the test material to cover about half of a petri dish during the irradiation period. Yet another possibility is to test the effectiveness of different colors of visible light in promoting photo repair. To do this, cover petri dishes with different colors of plastic filter material (borrowed from a school theater) or colored cellophane (purchased from a novelty store) after exposing the dishes to UV irradiation but before incubating the under lights. (The outcome of such experiments is difficult to predict here without knowing exactly what materials your student will test.)

Another good follow-up project is to have students use the library or the Web to investigate the chemical reactions by which chlorofluorocarbons, such as Freon, caused serious thinning of the ozone layer and created the ozone hole over the South pole. An examination of the international activities that led to bans on further production and use of such chlorofluorocarbons would be appropriate as well.

ANSWERS TO UV MUTAGENESIS WORKSHEET STUDENT PAGES 168-169

1. Draw pictures of what you observe on your dishes.
2. Describe the bacterial colonies on your dark-incubated control dish (0 seconds of UV). Then list and describe the differences among the bacterial colonies on each of the dark-incubated experimental dishes and those on the dark control dish.
Drawings and descriptions of dishes will vary but should indicate fewer colonies and more pink or white mutants as the UV exposure time increases. Students may also observe larger colonies on irradiated dishes than on the control.

3. Based on these observations, summarize the effects that UV irradiation has on *Serratia marcescens* bacteria.
Students should conclude that UV irradiation kills many bacteria and causes heritable changes in some of those that survive.

4. Now describe any differences among the bacterial colonies on each of the light-incubated dishes and those on the corresponding dark-incubated dish. (That is, compare the light-incubated control dish to the dark-incubated control dish, the light-incubated 30 sec. dish to the dark-incubated 30 sec. dish, etc.)
In principle, they should not see any significant differences between the dark and light control dishes, but they should see major differences in survival and mutant frequency among the survivors on the UV-irradiated dishes that were incubated in the light vs. the dark.

5. Based on these observations, summarize the effects that cultivation in visible light has on UV-exposed *Serratia* bacteria.
They should conclude that subsequent exposure to visible light somehow protects Serratia from the harmful effects of UV irradiation.

6. Can you formulate a hypothesis to account for such an effect of visible light?
It will be interesting to see what they will come up with here. It should be a good test of their ability to think scientifically. The answers to this question should provide a good starting place for an interesting class discussion, particularly if students are challenged to think of ways to test their hypotheses.

7. Do all of the bacteria on a dish appear to respond to UV and visible light in the same way? Why?
No. For example, many cells are killed, but some survive, and some of the survivors have visibly modified phenotypes, whereas others do not. This is because by sheer chance the UV will hit and damage different genes in different cells, and this will result in different phenotypes.

8. Do all of the mutations that you observe in *Serratia* after UV irradiation appear to be harmful? Explain.
No. For example, some of the irradiated bacteria have lost the ability to make the red pigment made by wild-type bacteria, but this does not appear to have affected their ability to grow and divide enough to produce a colony of offspring.

9. Is it possible that a mutation could be beneficial? Explain.
Some students may point out that some of the bacterial colonies on the irradiated dishes are larger than those on control dishes and then conclude that this indicates these bacteria have undergone a mutation that increases their growth rate. This can lead to an interesting discussion (see Hints and Troubleshooting).

CLOSURE

This exercise should provide your students with vivid evidence of the twin processes of environmental damage to DNA and DNA repair. Beyond that, however, it should provide a useful framework for stimulating them to think about how lifestyle decisions made by human beings today can influence their own genetic future and the genetic futures of their children, their grandchildren, and all other organisms with which we share the planet. When we and others carelessly discharge toxic substances from our homes, our automobiles, our power plants and our factories into rivers, air, and land, we may think that out of sight is out of mind. But we now have more than adequate evidence to appreciate that the mutagens and carcinogens that we release carelessly today may return tomorrow to adversely affect our genes or those of our grandchildren.

CHAPTER 3

How Genes and the Environment Influence Our Health

SECTION C

Is it Nature, or Is it Nurture?

Chapter 3: Section C Background

AS WE HAVE ALREADY mentioned, there is a potential danger in teaching genetics. The danger is that if we overemphasize the role of genes in controlling the traits of organisms, our students may adopt the mistaken concept of **genetic determinism**, which is the concept that organisms – including humans – are completely and irrevocably defined by their genes. If applied to bacteria or slime molds, genetic determinism (although scientifically unsound) probably has few serious social consequences. But when applied to human behavior, genetic determinism has had disastrous consequences, leading to the most severe forms of racism, the eugenics movement, forced sterilizations, and the ovens of Buchenwald. Even today genetic determinism rears its ugly head repeatedly in unexpected places and countless chilling ways.

If we did not all believe in the role of environment in shaping human behavior, we would not be in the education business.

This section provides a good opportunity to help students begin to strike a healthy balance in their view of genetics, by emphasizing the fact that every trait of every organism, including ourselves, is the product of some set of gene-environment interactions.

C.1

Albino Plants: A Model Gene-Environment Interaction

STUDENT PAGES 172-173

LESSON OVERVIEW

This exercise with tobacco seedlings provides a wonderful opportunity for your students to see the all-important concept given at the end of your Background section, namely, that "every trait of every organism, including ourselves, is the product of some set of gene-environment interactions."

The object of their investigation in this exercise will be one of the most important traits in the living world - the capacity to synthesize chlorophyll, the molecule upon which photosynthesis (and therefore the entire living world) depends. The seeds that the students will germinate were produced by crossing two tobacco plants that were heterozygous for a mutation at a locus encoding one of the enzymes required for chlorophyll synthesis. The wild-type allele encodes a functional enzyme and therefore is dominant with respect to the mutant allele. which does not encode a functional enzyme. However, the dominant allele, can not be expressed in the dark. Chlorophyll synthesis not only requires all of the genes encoding all of the enzymes in the pathway of chlorophyll biosynthesis, it also requires the correct environmental stimulus - light.

TIMELINE

This exercise requires little class time (particularly if the data analysis is assigned as homework), and thus it has a high benefit-to-cost ratio. Counting out the seeds and "planting" them on the agar plates requires only about five minutes the first day. Counting seedlings and recording data requires about five minutes on each of two later days.

Additional time will then be required if you ask the students to complete the optional Chi square analysis in class. However, if they performed the Chi square analyses in Chapter 2, this Chi square analysis could be assigned as homework. (On the other hand, if your students did not perform the Chi square analyses in Chapter 2 and you now wish to have them perform a Chi square analysis of their albino plant data, you will probably find it useful to introduce them to exercises E.2 and E.3 from Chapter 2 at this time.)

MATERIALS

For each group of three or four students:
- 1 petri dish with black agar
- 30 tobacco seeds
- a marking pen
- aluminum foil

ADVANCE PREPARATION

You can purchase the tobacco seeds from:
Carolina Biological
1-800-334-5551
www.carolina.com
Green:Albino Tobacco Seeds; catalog # BA-17-8400

It is a good idea to provide each lab group with a more-than-adequate sample of the seeds in a small envelope or a folded piece of paper rather than make them wait in line to count out their seeds from a central supply.

A day in advance, prepare dishes with black agar, as follows: To 500 ml of water in a 1 liter flask or beaker, add 15 grams of agar (any type of agar you have on hand will do). Heat on a burner or hot plate to bring the water to the boiling point and dissolve the agar. Allow to cool just enough that you can handle the container comfortably. Mix well and then stir in a tablespoon of powdered charcoal. Pour carefully into petri dishes and allow them to sit undisturbed until they have fully solidified. This recipe should be enough for about 25 dishes. The dishes do not need to be sterile.

Set up lights in the area where the dishes will be incubated. In order to minimize the differences in incubation conditions to which the "light" and "dark" dishes are exposed, have the students who have been assigned to the "dark" group wrap their dishes in foil and incubate them right next to the "light" dishes.

ANSWERS TO THE ALBINO PLANT WORKSHEET STUDENT PAGE 174

DATA FROM YOUR OWN PAIR OF GROUPS

Students should observe approximately a 3:1 ratio of green to white on the light dish and only white plants on the dark dish

DATA FROM THE WHOLE CLASS

Similarly, students should observe approximately a 3:1 ratio of green to white on the light dishes, and only white plants on the dark dishes

ANSWERS TO DATA ANALYSIS STUDENT PAGE 175

Use the data collected by the class as a whole to answer the following questions.

1. What ratio of green seedlings to white seedlings was present in the light-incubated seedlings on the first day of observation?
   Because students are instructed to use whole-class data, they should all have the same ratios.

2. What did you expect the ratio of green to white seedlings to be, given that this difference in color has a simple genetic basis? Explain.
   3:1 This is the ratio to be expected if the absence of green color is caused by a recessive allele at a single genetic locus.

3. Do you think that the difference between the exact ratio that you calculated for Question 1 and the expected ratio that you gave for Question 2 is significant? Explain
   Students may differ in their opinions regarding the significance of the deviation. However. if they performed exercises E.2 and E.3 in Chapter 2 they should say that one way to find out whether the difference is significant is to perform a Chi square analysis.

4. What ratio of green plants to white plants was present in the dark-incubated seedlings on the first day of observation?
   They should have observed no green seedlings following dark incubation.

5. Is this significantly different than the ratio of green plants to white plants that was present in the light-incubated seedlings on the first day of observation?
   Yes.

6. Formulate a hypothesis to explain the difference between the light-incubated and the dark-incubated seedlings.
   Students should postulate that light is required for chlorophyll synthesis.

7. How could your hypothesis be tested?
   By placing the dark-incubated plants in the light.

8. Compare the ratios of green to white seedlings that were observed in the dark-incubated seedlings on the first observation and the second observation:
   They should get a green:white ratio of approximately 3:1 on the second observation (in contrast to the absence of green seedlings that they should have seen on the first observation)

9 How do you account for any difference between these two ratios?
   Chlorophyll synthesis requires light, so you can't analyze genes involved in controlling chlorophyll synthesis in seedlings that have not been exposed to light.

10. What overall conclusion can you draw from the data collected in this experiment?
   Both genes and environment are important in determining the phenotype of an organism.

AN OPTIONAL STEP: CHI-SQUARE ANALYSIS ANSWERS STUDENT PAGE 177

1. Following the instructions for How to Perform a Chi-Square Test on Any Data Set, (Chapter 2, Section E.3) and using the data collected by the class as a whole, calculate χ^2 and p for the light-incubated seedlings on the first day of observation.
   It is hoped that the whole-class data will yield a value of p greater than 5%.

2. Based on the value of p that you obtained, do you think that the class data for the light-incubated seedlings are consistent with the hypothesis that seedling color is determined by a pair of alleles that exhibit a simple dominant-recessive relationship? Explain.
   Students should use the 5% cut-off rule to determine the significance of the deviation between their expected and observed ratios: If they get a p value greater than 5%, this indicates that such large deviations can be expected to occur more than 5% of the time by chance alone and therefore that their data are consistent with the hypothesis used to make the predictions.

3. Repeat the calculation of χ^2 and p for the light-incubated seedlings on the first day of observation, using only the data collected by your own group.
   It may be that some students will obtain p values smaller than 5%, owing to their small sample size.

4. Based on the value of p that you obtained this time, do you think that your own data are consistent with the hypothesis that seedling color is determined by a pair of alleles that exhibit a simple dominant-recessive relationship? Explain.
   See answer to Question 2.

5. Which data set gave you the higher p value, the class data or your own data? Explain.
   This could go either way because of the statistics of small numbers. Some groups may get a larger p value with their own data than with the class data, and other groups may get a smaller p value.

6. Do you think you need to perform a χ^2 test to determine whether the data that your class collected with respect to the dark-incubated plants during the first observation are consistent with the proposed hypothesis? Explain.
   No. It should be obvious by inspection that the data are inconsistent with the hypothesis.

C.2

Heart Disease: a Personal Gene-Environment Interaction

STUDENT PAGES 178-181

LESSON OVERVIEW

This exercise attempts to demonstrate the personal relevance of the principle illustrated by the previous exercise – namely that gene-environment interactions determine one's phenotype. Students will perform a simulation that allows them to see how their genes and their lifestyle will interact to determine their chance of developing heart disease.

Although heart disease is the example chosen for illustration here, most serious human diseases (such as cancer, diabetes, and schizophrenia) resemble heart disease in that they are **multifactorial**. That is to say, the probability that one of these diseases will strike any given individual – and the severity of the symptoms if it does occur – is determined by a number of interacting genetic and environmental factors.

TIMELINE

This exercise and discussion requires 50 minutes to complete.

MATERIALS

For each group of four or five students:
- 4 labeled envelopes containing Environmental Risk Cards
- 2 containers each containing 12 poker chips or other objects in three colors

ADVANCE PREPARATION

Photocopy, cut apart, and separate by category the Environmental Risk Cards, which are found on page T196. You will need a minimum of one copy of the page for every five students in your class, and lamination will make the cards much more durable. Place the cards printed side down in envelopes that are labeled *A - Body Weight, B - Diet, C - Smoking,* and *D - Exercise.*

Prepare two containers with objects of similar size and shape in three different colors. (For example, red, white and blue poker chips, although pieces of colored paper will do.) Label one container *Father's Genes* and the other container *Mother's Genes*. Place four objects of each color in each container. Make sure that the three colors are well-mixed.

PROCEDURE

Pass out envelopes containing Environmental Risk Cards to each group. Each student should take one card from each envelope and record the results on the work sheet.

Pass the containers with colored objects to each group. Each student should draw three objects from each container with eyes closed, record the colors on the work sheet, then return the objects to the bowl and mix them up before passing the bowl on. Make it clear that the intent is to simulate which allele each student inherited at each of three different loci that affect heart health. (The three different colors indicate three different alleles that can be present at each of those three loci.)

After all students have drawn and recorded three Mother's Genes and three Father's Genes on their work sheets, inform them of the genetic risk that you have assigned each color. The recommended values are 0, 2 and 4. (For example, white = a genetic risk of 0, blue = 2, and red = 4.)

HINTS AND TROUBLESHOOTING

After students have completed their work sheets, you might wish to go around the room, asking students to read out the values they have calculated for the percentage of their total heart disease risk that is due to genetic factors. (You may even wish to tabulate and average the numbers.) This exercise was designed so that the average genetic risk would be about 50% of the total risk. This ratio is arbitrary but probably not too far from reality: few individuals have such a high genetic risk that they are extremely likely to develop heart disease despite a relatively healthy lifestyle, and, similarly, few individuals can lead very unhealthy lifestyles and not develop heart disease because of a very low genetic risk.

Some of the points that you might wish to bring out during the discussion might include:

- The numbers that are used in this exercise for risk factors are for illustrative purposes only. While they are consistent qualitatively with current understanding of certain heart disease risk factors, it should not be assumed that they accurately reflect the actual numerical risks.
- Only four lifestyle factors have been considered here. Although these may be among the most important factors, they are not the only factors affecting the risk of heart disease. Others the students may be able to think of are stress, alcohol and drug abuse. One nongenetic risk factor they probably will not think of is testosterone, which is the reason heart attacks are more common in men than in women.
- Similarly only three loci, with only three alleles per locus (represented by the three different colors), have been considered in this simulation. In reality numerous loci appear to influence the development of heart disease. Moreover, these differ much more in their relative importance than the three loci considered here; some have only a modest effect, whereas others have a very pronounced effect on the probability that heart disease will strike.

- Furthermore, this simulation is based on the assumption that all genetic and lifestyle risks are additive. This is known to be a false assumption; some risks are known to interact in a multiplicative fashion. For example, dietary factors such as saturated fat and cholesterol intake assume much greater importance in individuals with genetic abnormalities in lipid metabolism than they do in the rest of the population. *
- Even with a very high risk score, it is seldom the case that one is certain to get heart disease. Nor does a very low risk score guarantee that one will never have heart disease. Chance plays a part. For example, in a pair of identical twins with virtually identical lifestyles, it is sometimes the case that one twin will suffer a heart attack and the other will not.
- Students who want more information about their personal risk of heart disease should be referred to their family doctor or other health care professional. Your local chapter of the American Heart Association can provide useful information and handouts for students.

*If time permits, you may find it useful to go into more detail about the factors affecting blood cholesterol levels, in order to make the concepts in this paragraph and the preceding one seem less abstract. The following should provide more than enough material for organizing such a discussion.

Most people now realize that diet (and particularly the amount of saturated fat eaten) is an important determinant of blood-cholesterol levels, and they know that people with high blood-cholesterol levels have a high heart attack risk. But few people realize how many different genes also contribute to determining their blood-cholesterol levels. Our blood cholesterol level is influenced not only by the amount of fat we eat, by also the alleles we happen to have inherited from our parents at each of the several dozen loci that encode proteins involved in various aspects of fat metabolism. Individually, most common alleles at most of these loci have only modest positive or negative effects on blood cholesterol levels. Nevertheless, they interact, and certain combinations of alleles at several of these loci can result in extremely large differences in blood cholesterol levels between individuals who have extremely similar diets and life styles. But in addition to these many "minor-effect" loci, there several loci at which mutant alleles can have a really major, life-threatening impact on blood cholesterol levels. The most important of these genes and its product will be discussed below, but in order to understand why it is so important, we need to understand certain aspects of the chemistry of blood cholesterol, and why elevated cholesterol levels are associated with heart disease.

Because cholesterol is a fatty substance that is not water soluble, it can only remain suspended in the blood stream if it is in the form of a complex with carrier-protein molecules known as **lipoproteins.** Cholesterol molecules that accidentally slip out of such a complex precipitate out of the blood to form waxy deposits on the walls of blood vessels. Such cholesterol deposits lead to **atherosclerosis,** or hardening of the arteries, which leads in turn to a greatly elevated risk of heart disease. The most common type of heart attacks are caused by **coronary artery disease,** in which atherosclerosis of the coronary arteries reduces the blood flow to the wall of the heart so much that the heart becomes starved for oxygen and quits beating rhythmically.

Most of the cholesterol in the blood is carried as a complex with **low-density lipoprotein (LDL).** This LDL-cholesterol complex **(LDL-C)** is what physicians frequently call "bad cholesterol," because a consistently high level of LDL-C is what leads to cholesterol deposits in blood vessels and atherosclerosis. This is because a single LDL molecule can carry as many as 1500 cholesterol molecules, but these molecules are held rather loosely, so that they tend to fall off and form cholesterol deposits. Thus, the more LDL–C that is circulating, the more likely it is that some cholesterol molecules will fall off the complex. In contrast, the remaining 10-25% of the blood cholesterol (the **HDL-C,** which is carried by **high-density lipoprotein**) is called "good cholesterol," because HDL is capable of picking up cholesterol molecules that have been deposited on blood vessel walls, thereby counteracting the negative effects of LDL-C, and slowing the development of atherosclerosis.

If cholesterol is such nasty stuff, why do we have it in our blood at all? Why has natural selection not acted to remove cholesterol from the blood stream? The answer is simple: cholesterol is an essential component of all of our cellular membranes, and thus every one of our cells must have a supply of cholesterol. Surprising as it may seem at first, it is the "bad cholesterol" (LDL-C), not the "good cholesterol" (HDL-C), that cells tap into when they need cholesterol for making new membranes. When cells anywhere in the body are stimulated to grow or to repair their membranes, they make a plasma-membrane protein called **low-density-lipoprotein receptor (LDL-R).** LDL-R then traps circulating LDL-C complexes and causes them to be brought into the cell, whereupon the LDL is degraded while the cholesterol is used to make new membranes. When the cell has all the cholesterol it needs for membrane synthesis, it degrades its LDL–R, terminating the accumulation of cholesterol.

Whereas most body cells make LDL–R only when they are making new membranes, liver cells normally have LDL–R in their plasma-membranes at all times. This permits the liver to act as the garbage disposal for excess LDL-C in the blood. The more LDL–C in the blood, the more the liver takes up and destroys, thereby playing a critical role in regulating the amount of LDL–C in the blood.

Thus, both the uptake of LDL–C by cells that need cholesterol for growth, and the uptake of excess LDL–C by liver cells that are prepared to destroy it, requires a functional LDL–R molecule. Not surprisingly, therefore, a mutation in the gene encoding the LDL–R protein can markedly decrease the ability of the liver to take up and destroy LDL–C, which results in a marked increase in LDL–C in the blood, and the disease known as **familial hypercholesterolemia,** or **FH.** FH is one of the most common hereditary human diseases, affecting one person in 500 (which means it is much more common than cystic fibrosis or sickle cell anemia, for example). Why is it so common? The answer is: because it is inherited as an autosomal dominant condition, which means that everyone who has one mutant gene has FH symptoms. This is because it takes only one mutant gene to decrease the amount of LDL–R on liver cells by half, and this is all it takes to double the level of LDL–C in the blood, moving it into the danger zone. Moreover, with only half as many LDL–R molecules in the liver to remove excess cholesterol from the blood, the LDL–C levels of people with FH are much more sensitive to differences in dietary fat intake than are the LDL–C levels of people with two normal LDL–R genes. Thus the occasional fatty steak that can be well tolerated by other individuals can be life threatening for someone with FH. Moreover, from the moment of birth the LDL–C levels of babies with FH average twice as high as those of babies lacking LDL–R mutations, and thus development of atherosclerotic lesions begins in early infancy, particularly if the infants have a high-fat diet.

Perhaps the statistic that indicates most clearly the importance of the LDL–R gene in human health is the fact that although the frequency of FH in the general population is only 1 in 500, the frequency of FH among those who suffer serious heart attacks is 1 in 20. Putting it differently, people with FH are 25 times as likely to have a heart attack as people without FH. Men with FH may have fatal heart attacks before age 40, and 85% of them will have heart attacks before age 60. Women with FH have nearly as great risks of serious heart attacks in the long run, but their peak risks are delayed by several years. (This difference is attributed to the effect of testosterone on cholesterol metabolism.)

If FH is so serious, why has it not been eliminated from the population by natural selection? Again the answer appears straightforward. Until blood-cholesterol assays became fairly routine, most people were unaware that they had FH until they had a heart attack, by which time their children had been born and their mutant LDL–R gene had been passed on. Even though most people now appear to be aware of the importance of controlling their blood cholesterol levels, a study performed in 2000 found that only one fourth of the people between the ages of 30 and 50 who had FH had been diagnosed before the study was performed. Natural selection is powerless to eliminate heritable traits that arise after the reproductive years are over, no matter how detrimental to later health those traits may be.

Although natural selection does not act to prevent reproduction by FH heterozygotes, it certainly does so for FH homozygotes. The unfortunate one human being in 1,000,000 who is born with two mutant LDL–R genes has LDL-C levels that are so high that heavy cholesterol deposits begin to form almost immediately – not only in blood vessels, but also in skin, in hands, feet and eyes, and various other organs throughout the body. Such children may suffer fatal heart attacks before they are two years old, and they seldom live to puberty, even with strict dietary supervision and the best medical care available.

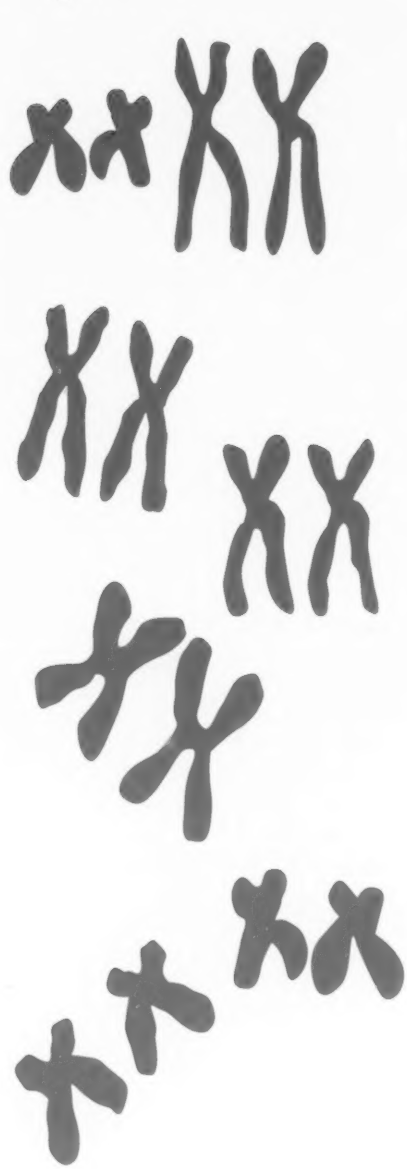
ACKNOWLEDGMENTS

This exercise was adapted from Lesson 3 in *Health in the Year 2100: What's Heredity Got to Do With It?*, a high-school genetics-curriculum unit developed at the University of South Dakota School of Education.

CLOSURE

The lessons from this exercise and the preceding one both point to the idea that genes and environment interact to determine phenotype. However, it is hoped that through this exercise your students will have come to appreciate that this is not just an abstract scientific generalization to be learned for a test but a statement about the real world that could affect their own lives in many important ways.

Maintain desirable body weight at all times SCORE = 0 **A**	Sometimes up to 20 pounds overweight SCORE = 2 **A**
Usually 10-20 pounds overweight SCORE = 3 **A**	Always 20-30 pounds overweight SCORE = 4 **A**
Always at least 50 pounds overweight SCORE = 6 **A**	Strict vegetarian SCORE = 0 **B**
Eat chicken or fish, but no beef SCORE = 2 **B**	Eat beef or pork once or twice a week SCORE = 3 **B**
Eat beef or pork most days SCORE = 4 **B**	Eat beef or pork at least once a day SCORE = 5 **B**
Never smoke SCORE = 0 **C**	Smoke less than five cigarettes a week SCORE = 2 **C**
Smoke one to four cigarettes per day SCORE = 4 **C**	Smoke about one pack of cigarettes every day SCORE = 6 **C**
Smoke more than one pack of cigarettes every day SCORE = 8 **C**	Vigorous exercise every day SCORE = 0 **D**
Vigorous exercise most days SCORE = 1 **D**	A little bit of exercise every day SCORE = 2 **D**
Exercise every other day or so SCORE = 3 **D**	Exercise once a week or less SCORE = 6 **D**



CHAPTER 3

How Genes and the Environment Influence Our Health

SECTION D

What Are Some of the Features of "Simple" Genetic Diseases?

Chapter 3: Section D Background

THE VAST MAJORITY OF human beings are born healthy. Sadly, however, many are not. About one child in twelve in this country is born with a birth defect that will cause significant health problems by no later than age seven. Some genetic abnormality plays an important role in the development of the disease symptoms in a majority of such **congenital defects**. The term **genetic disease** is applied in all such cases, despite the fact that it is known that environmental factors usually play important roles in determining whether or when disease symptoms will appear and/or how serious those symptoms will be. More than 4,000 diseases in which a mutation of a single gene is a causative factor are now recognized. These are the so-called **single-gene diseases**. Currently, 25-30% of the patients in a typical metropolitan children's hospital will be individuals suffering from some such disease.

There may be a tendency to think of the parents of children who suffer from such a disease as "people with bad genes." Such an attitude clearly cannot be justified, however. On average, each of us is a carrier for mutations in about twenty genes, each of which would have caused us to have a serious disease had we received two mutant copies of one of those genes instead of just one. One of your twenty mutations also could cause you to be one of those unfortunate parents with a very sick child if you have a child with another person who carries one of the same mutations. The only reason that so few children suffer serious genetic disease is that each of the mutations we carry is so rare that we are extremely unlikely to meet and fall in love with someone who has mutations in the same genes.* But it could happen, of course. So serious genetic diseases are not so much the result of having bad genes as having bad luck.

This section deals primarily with single-gene diseases. Its focal point is Section D.5, in which students are challenged to learn as much as they can about one such disease and produce an informative pamphlet about that disease. (This pamphlet should be of the type that might be used by a genetic counselor.) The rest of the section provides background information that will help your students understand some general principles regarding genetic diseases and certain kinds of specific information that they may encounter as they do their research.

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*The exception to this rule comes if one marries a close relative. Recognition of this fact from time immemorial is why most societies have banned marriage between cousins or more closely related individuals.

D.1

Some "Simple" Heritable Defects

STUDENT PAGES 184-185

LESSON OVERVIEW

This lesson is a student reading designed to introduce a few examples of familiar human conditions caused by a gene mutation that results in a single protein being defective.

TIMELINE

It will take an average student 5 minutes to read this material. You should discuss the table on S185 in class, as most students will have heard of many of the conditions listed, but will likely know little about the protein affected.

D.2

Phenylketonuria (PKU) Illustrates the Complexities of Some "Simple" Genetic Diseases

STUDENT PAGES 186-187

LESSON OVERVIEW

This lesson is a student reading designed to illustrate how "simple" single-gene diseases often involve complex interactions among multiple genetic and environmental factors.

TIMELINE

It will take an average student 5 minutes to re[illegible] is material. It could be assigned as homework.

D.3

The Special Inheritance Patterns of Sex-Linked Mutations STUDENT PAGE 188

LESSON OVERVIEW

In this lesson, students will learn about the unusual inheritance patterns that accompany a mutation of a gene that is located on the X chromosome. Students will also deduce some of the special consequences arising from those mutations.

TIMELINE

The reading and work sheet require 50 minutes to complete.

ANSWERS TO SEX-LINKED MUTATION WORK SHEET STUDENT PAGE 189

1. If a woman is a carrier for a mutation causing a sex-linked disorder, what is the chance (in percentages) that one of her sons will have the disorder? Explain.
   50% Each of her sons will inherit one of her X chromosomes, and there will be a 50-50 chance that it will be the one carrying the mutant allele.

2. If a woman who is a carrier for a sex-linked disorder already has one son who has the disorder, what is the chance that if she has a second son he will also have the disorder? Explain.
   50% The probability that each son will inherit the mother's mutant allele is 50%, regardless of which allele any of his brothers has inherited from her.

3. If a man has a sex-linked disorder, what is the chance that he will pass it on to one of his sons? Explain.
   0 % Sex-linked mutations are carried on the X chromosome, and sons never inherit an X chromosome from their father.

4. If a man has a sex-linked disorder, what is the chance that one of his daughters will be a carrier for that disorder? Explain.
   100% Every girl inherits an X chromosome from her father, and the only X chromosome a man with a sex-linked disorder has is the one carrying the mutation.

5. If a man has a sex-linked disorder, what are the chances that one of his grandsons will inherit that disorder? Explain
   0 or 50% The sons of his sons will have a 0% chance of inheriting his disorder, but the sons of his daughters will have a 50% chance.

6. It has been postulated that a condition known as hairy ears is caused by a mutation of a gene on the Y chromosome. Assuming this is true, what is the chance that one of the sons of a man with hairy ears will inherit the hairy-ear mutation? Explain.
   100% Every boy inherits his father's Y chromosome.

7. What is the chance that one of the daughters of the man referred to above will pass the hairy-ear mutation on to one of her sons? Explain.
   0% Girls inherit their father's X chromosome, never his Y chromosome, so his daughters do not get a copy of his hairy-ears chromosome to pass on.

D.4

Investigating Human Genetic Diseases

STUDENT PAGES 190-192

LESSON OVERVIEW

This exercise is designed to help your students study particular human diseases on their own and in detail. Then they are challenged to synthesize what they have learned and produce an informative pamphlet that is of the sort a genetic counselor might give to an individual or family afflicted with the disease in question.

TIMELINE

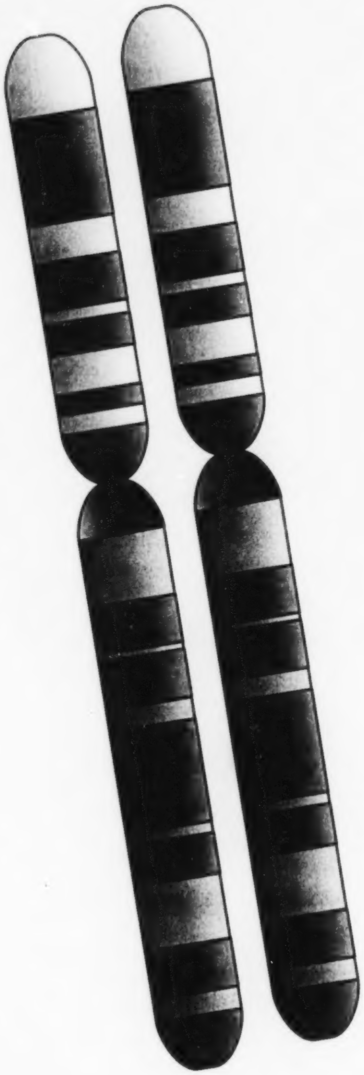
It is recommended that students be given a week to perform their research and produce a pamphlet. You will need to decide how much class time will be devoted to this and how much you will expect to be done as homework. It usually requires at least three class periods (50 minutes each) for students to do adequate research and produce the pamphlet during class time.

PROCEDURE

You need to decide whether to assign a topic to each student, allow each of them to pick a topic from the lists in this section and Section D.1, or have each of them draw the name of a disease from a hat. There are substantial advantages to the latter method: If students are allowed to choose their own topics, they often will all end up studying one of a handful of most familiar diseases, such as PKU, cystic fibrosis, or sickle-cell anemia. On the other hand, when the teacher assigns the topics, some students get disgruntled if they believe that they have been assigned a "tough" or "boring" topic, whereas a friend has been assigned an "easy" or a "cool" one.

HINTS AND TROUBLESHOOTING

Many computer word processing programs (such as Microsoft Word and Claris Works) have a multicolumn option that allows students to design an attractive trifold pamphlet.



CHAPTER 3

How Genes and the Environment Influence Our Health

SECTION E

How Does a Genetic Counselor Detect Mutant Genes?

Chapter 3: Section E Background

DUCHENNE MUSCULAR DYSTROPHY (DMD), which is the subject of the following exercise, is a relatively common sex-linked disease. It affects about 1 boy in 3000, most of whom appear to be healthy until age 4 or 5, whereupon they begin to develop muscular weakness. Frequently, the first symptoms are problems with running and climbing stairs. Affected individuals are usually wheelchair-bound before they reach their teens and few survive into their twenties, most frequently dying from lung or heart failure. Fewer than 10% of carrier females exhibit any muscular weakness as a consequence of having one mutant allele, and female homozygotes are extremely rare, since very few affected males ever become fathers. There are currently no effective treatments for this disease.

DMD can result from any one of a variety of mutations in the gene coding for **dystrophin**, an important structural protein in the muscle, heart, and brain. (Dystrophin's essential role in the brain is pointed to by the fact that patients with DMD often show a decline in IQ scores as the disease progresses.) A milder form of the disease, called Beckers muscular dystrophy results from mutations that cause production of a partially functional dystrophin molecule.

The dystrophin gene is the largest human gene that has been studied to date and occupies almost 2% of the entire X chromosome. Perhaps because of its size, this gene has an extremely high mutation rate, and nearly one third of all cases of DMD are the result of new mutations that occurred by chance during formation of the egg from which the affected boy developed. In such cases, the mother is not a carrier for DMD and is very unlikely to have a second child with a defective DMD allele. However, because very few boys with DMD ever live long enough – or are healthy enough – to produce children, new mutant DMD alleles that show up in boys usually disappear from the population in one generation.

New DMD mutations that show up in girls persist much longer, on average, because such girls become carriers, who have a 50% chance of passing their mutant gene on to each of their offspring. Moreover, because girls receive an X chromosome from each parent, they're about twice as likely as boys to end up with a novel DMD mutation – one that occurred during formation of either the egg or the sperm from which they received their genes. It is estimated that an average genetically normal male produces a sperm cell with a new mutation in the dystrophin gene every 10-11 seconds. (As high as this mutation rate may sound, the rate of sperm production in a healthy male is so enormous that only a tiny fraction of all sperm carry a new DMD mutation.)

Most dystrophin gene defects resulting in DMD are **deletions** (the absence of some normal portion of a chromosome) of varying sizes. These deletions are the basis for many of the available diagnostic tests. Frequently, DNA samples from patients and family members are analyzed by **PCR amplification** of several different portions of the dystrophin gene where deletions are known to occur, followed by separation of the resulting DNA fragments on an agarose gel. (PCR amplification is explained in the next section.) Individuals with deletions will either lack certain DNA bands or will exhibit smaller bands than family members without the defect.

BACKGROUND REFERENCES

Mange, A. P. and Mange, E. J. Genetics: Human Aspects. 36-40. Sunderland, Mass.: Sinauer Associates, 1990.

National Institute of Health. Understanding Gene Testing. (Bethesda, MD: NIH. 1995), Pub. 95-3905.

HOW PCR IS USED TO AMPLIFY A GENE FRAGMENT OF INTEREST

The polymerase chain reaction (**PCR**) referred to above is one of the most important, most powerful and most widely used techniques in modern biology. PCR is used routinely for a wide range of purposes by research biologists and genetic counselors (as is simulated in the following exercise). It also has become the most important method used by law enforcement agencies for personal identification. With it, a single hair or a single drop of blood found at a crime scene can be used to trap the guilty or free the innocent. Information in *Jurassic Park* notwithstanding, PCR has not yet been used to bring back the dinosaurs, nor is it likely to. However, PCR has been used to examine DNA sequences of tiny bits of plants and animals that lived long ago – including insects trapped in amber for more than 100 million years. Indeed, PCR has become so important in many areas of biology and medicine that Kary Mullis was awarded the Nobel Prize in Chemistry for inventing it.

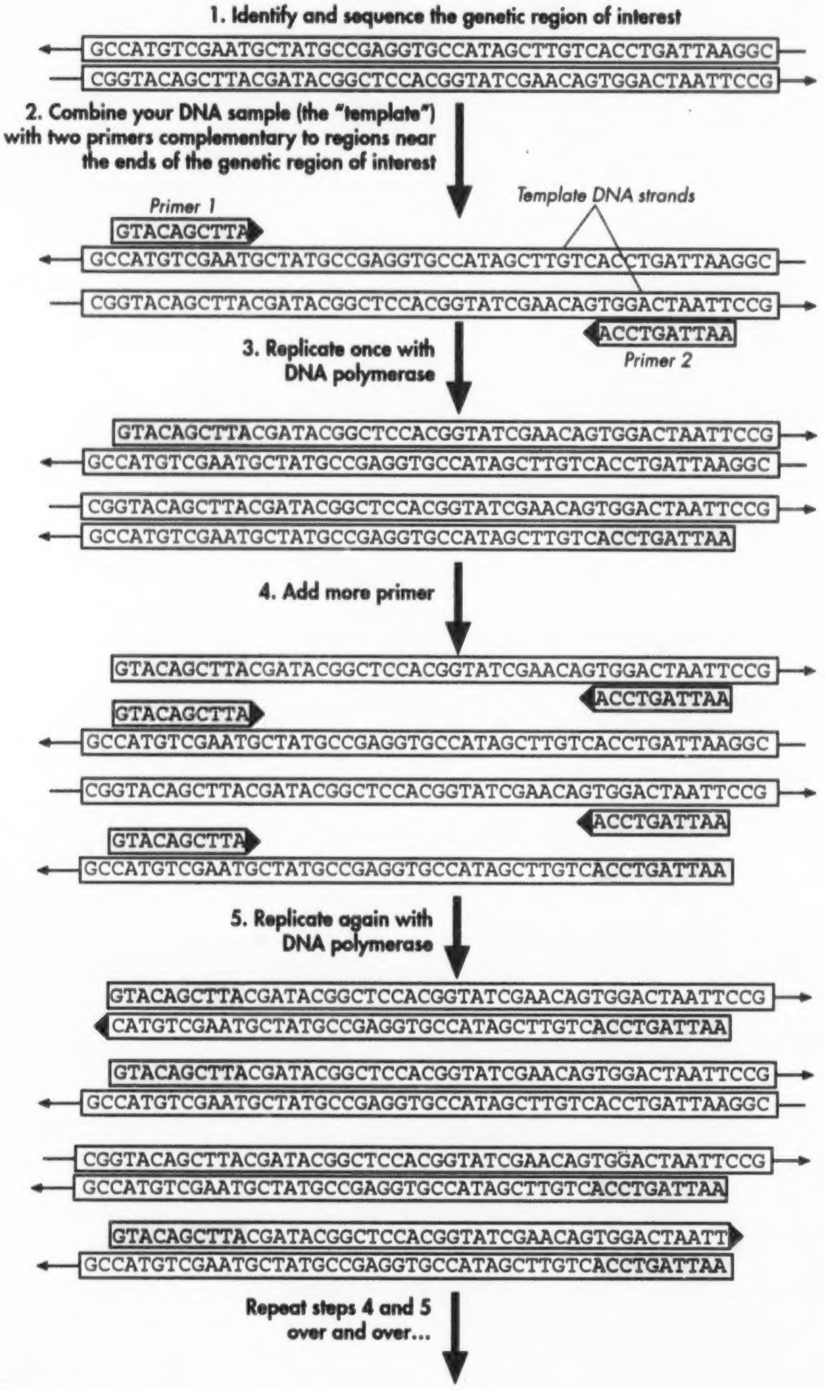
PCR is based on one simple but important fact about **DNA polymerase**, the enzyme that replicates DNA in cells before each round of cell division. This fact is that in order for DNA polymerase to replicate any target DNA molecule (which is called its **template**), it must have a short piece of nucleic acid, called a **primer**, that is complementary in its base sequence to part of the template. The primer base-pairs to the template and acts as the starting place for DNA synthesis. DNA polymerase then functions by adding nucleotides to the primer in the sequence that is specified by the template.

In living cells, a whole set of other proteins are required to find the location on a chromosome where replication is to begin and to make a primer that DNA polymerase can use to get started. The details of how this is accomplished need not concern us here. What is important is to recognize that if DNA polymerase has a primer and a template it can begin replicating the DNA. But if DNA polymerase has no primer, it is incapable of copying DNA. Therefore, one can get DNA polymerase to copy just exactly the part of a template DNA molecule that one is interested in by providing it with primers that define the desired starting places on both strands of the double-stranded molecule of interest. (See the diagram on the next page.)

Of course, one must know the DNA sequence in the region of interest in order to design useful primers. In the diagram, the primers shown are ten nucleotides long (and with pointed ends indicating where DNA polymerase will add nucleotides). In actual practice longer primers must be used to assure that they will base pair with – and initiate DNA replication from – only the desired sites in the template DNA molecules. That is because a site complementary to a primer only 10 nucleotides long will occur by chance about once in every 4^{10} (equal to about one million) base pairs, or about three thousand times in the human genome, which is 3×10^9 base pairs long. In contrast, a site complementary to a primer 25 nucleotides long will occur by chance only about once in every 4^{25}, or 10^{15}, base pairs.

In the diagram, the starting DNA sample is represented by a sequence of bases in the region of interest superimposed on an arrow (which is meant to indicate that the template goes on and on). In the first round of replication, only the initial DNA serves as a template. But then in the second round the new DNA molecules can also be used as template. So in each round of synthesis the number of template molecules used, and the number of new molecules produced is doubled. This is where the chain reaction part of the term *PCR* comes from. In 30 rounds of replication, 2^{30} (more than a billion) molecules of the gene of interest are produced for every starting double-stranded molecule present in the original sample. This process produces more than enough of the DNA needed to allow for its study in a variety of ways. This includes gel electrophoresis, the process your students will simulate.

POLYMERASE CHAIN REACTION DIAGRAM


1. Identify and sequence the genetic region of interest
GCCATGTCGAATGCTATGCCGAGGTGCCATAGCTTGTCACCTGATTAAGGC
CGGTACAGCTTACGATACGGCTCCACGGTATCGAACAGTGGACTAATTCCG
2. Combine your DNA sample (the "template") with two primers complementary to regions near the ends of the genetic region of interest
Primer 1
GTACAGCTTA
Template DNA strands
GCCATGTCGAATGCTATGCCGAGGTGCCATAGCTTGTCACCTGATTAAGGC
CGGTACAGCTTACGATACGGCTCCACGGTATCGAACAGTGGACTAATTCCG
ACCTGATTAA
Primer 2
3. Replicate once with DNA polymerase
GTACAGCTTACGATACGGCTCCACGGTATCGAACAGTGGACTAATTCCG
GCCATGTCGAATGCTATGCCGAGGTGCCATAGCTTGTCACCTGATTAAGGC
CGGTACAGCTTACGATACGGCTCCACGGTATCGAACAGTGGACTAATTCCG
GCCATGTCGAATGCTATGCCGAGGTGCCATAGCTTGTCACCTGATTAA
4. Add more primer
GTACAGCTTACGATACGGCTCCACGGTATCGAACAGTGGACTAATTCCG
ACCTGATTAA
GTACAGCTTA
GCCATGTCGAATGCTATGCCGAGGTGCCATAGCTTGTCACCTGATTAAGGC
CGGTACAGCTTACGATACGGCTCCACGGTATCGAACAGTGGACTAATTCCG
ACCTGATTAA
GTACAGCTTA
GCCATGTCGAATGCTATGCCGAGGTGCCATAGCTTGTCACCTGATTAA
5. Replicate again with DNA polymerase
GTACAGCTTACGATACGGCTCCACGGTATCGAACAGTGGACTAATTCCG
CATGTCGAATGCTATGCCGAGGTGCCATAGCTTGTCACCTGATTAA
GTACAGCTTACGATACGGCTCCACGGTATCGAACAGTGGACTAATTCCG
GCCATGTCGAATGCTATGCCGAGGTGCCATAGCTTGTCACCTGATTAAGGC
CGGTACAGCTTACGATACGGCTCCACGGTATCGAACAGTGGACTAATTCCG
GCCATGTCGAATGCTATGCCGAGGTGCCATAGCTTGTCACCTGATTAA
GTACAGCTTACGATACGGCTCCACGGTATCGAACAGTGGACTAATT
GCCATGTCGAATGCTATGCCGAGGTGCCATAGCTTGTCACCTGATTAA
Repeat steps 4 and 5 over and over...

BEST COPY AVAILABLE

E.1

Detecting the Duchenne Muscular Dystrophy (DMD) Mutation

STUDENT PAGES 194-199

LESSON OVERVIEW

In this electrophoresis exercise, your students will pretend to be technicians in a genetic counseling lab. They will simulate the technique that would be used in such a lab to determine which family members might share the mutant dystrophin gene that has caused one of the Smith boys to develop Duchenne muscular dystrophy. To circumvent all sorts of logistic problems, in this simulation we will use two dyes with different electrophoretic mobilities to simulate two PCR fragments of different lengths - the normal allele and the DMD allele of the dystrophin gene.

Key concepts explored in this activity include a) electrophoresis as a way to separate charged molecules from one another, b) DNA analysis as a way to detect genetic abnormalities, c) X-linked genetic diseases, d) genetic counseling, and e) the personal implications of DNA analyses. It is an excellent complement to Exercise D.4, in which the students will prepare a pamphlet for use by a genetic counselor. Thus, you should schedule this activity for the period of time during which your students will gather the information for their pamphlets.

TIMELINE

There are at least three different ways you can schedule this exercise. Options 1 and 2 require that you have at least as many gel combs and at least half as many gel casting trays as you will have lab groups in all of your classes combined. If you have more than one class but only enough gel casting trays for one class, Option 3 is your only choice. Option 1 takes the least class time but requires more advance preparation on your part.

Option 1

You pour the gels in advance; the students complete the rest of the exercise, including analysis and discussion, in one class period (50 minutes).

Option 2

Day 1: One period is devoted primarily to discussion of various topics related to this exercise, such as the nature of sex-linked diseases in general and DMD in particular, the principles of electrophoresis, and genetic diagnosis and counseling. The students also pour their gels, wrap them in plastic wrap, and store them in the refrigerator overnight.
Day 2: Students load their gels (5 minutes), run the electrophoresis (15 minutes), and then record, analyze, and discuss their results (30 minutes).

Option 3

Day 1: Students perform the entire lab exercise in one class period, including pouring their gels, loading and running them as soon as the agarose has solidified, and recording the results on their work sheets. This will take most of a 50 minute period, but there will be two 10-15 minute intervals (while the gels are solidifying and while the electrophoresis is occurring) when some discussion will be possible.
Day 2: Students analyze and discuss their results.

MATERIALS

For each class:
- electrophoresis power supplies
- gel electrophoresis chambers

For each group of students (group size to be determined by equipment availability):
- 1 precast agarose gel, or 1 gel-casting tray plus masking tape, 1 or 2 gel-casting combs and 50 ml of 0.8% agarose in water
- a small container of tap water
- 1 20 µl micropipettor
- 6 dye samples labeled A-F
- 6 pipette tips

The number of electrophoresis chambers and power supplies that you have available will determine the maximum number of groups that can perform this exercise at once. A power supply that will run two gel electrophoresis chambers at once works well for this exercise. The number of groups that can run samples simultaneously with one such power supply is increased to four if extra combs are available. This will allow two sets of samples to be run in each gel. The combs and trays are inexpensive relative to the cost of the rest of the equipment, and they greatly increase flexibility. If you will be running two sets of samples per gel, you or the students who are preparing the gel will need to place two combs in each tray before pouring the agarose. One comb should be placed about 1 cm from one end and the second comb should be placed just beyond the middle of the tray.

The above mentioned materials can be ordered from:
Carolina Biological
(800) 334-5551
www.carolina.com
Electrophoresis Power Supply- catalog # BA-21-3672
Gel Electrophoresis Chamber- catalog # BA-21-3668
Extra Comb- catalog # BA-21-3666
Extra Gel Casting Tray- catalog # BA-21-3667

Agarose can be ordered from:
Carolina Biological
Agarose- catalog # BA-21-7080

Sigma Chemical Company
(800) 325-3010
www.sigma/aldrich.com/order
Agarose- catalog # A0169
Bromphenol blue and xylene cyanole can be ordered from:
Sigma Chemical Company
Bromphenol Blue- catalog # B0126
Xylene Cyanole- catalog # X4126

ADVANCE PREPARATION

1. **Prepare 0.8% agarose solution, 50 ml per group.** The following recipe is for 200 ml (4 groups):
   - To 200 ml water in a 500 ml flask add 1.6 grams agarose.
   - Cap flask with foil and heat carefully on a hot plate, or cap with plastic wrap and heat carefully in a microwave oven for 3-5 minutes.

   Safety Note: Agarose solution can superheat and either boil over during heating or erupt violently when the flask is touched. Handle hot agarose very carefully, wearing safety goggles and heavy gloves.

   - Swirl flask and make sure that all agarose has dissolved.
   - If gels are to be poured right away, cool flask to about 60°C before pouring.
   - If gels are to be poured later the same day, hold flask in a 60°C water bath until use.
   - If gels are to be poured another day, store solution covered and refrigerated. (Keeps for several weeks.) Then, well in advance of scheduled use, reheat agarose carefully on a hot plate or in a microwave oven until it is completely melted. Then bring it to about 60°C before pouring gels.

   If you will be pouring the gels yourself, follow the instructions given on the student pages for this exercise. When the gels have solidified, wrap each one in plastic wrap to prevent it from drying out.

2. **Prepare stock solutions of bromphenol blue and xylene cyanole.** In this exercise we will simulate the DNA samples of the various Smith family members with a pair of dyes, bromphenol blue and xylene cyanole. Bromphenol blue (BB) travels faster in an agarose gel, so it will be used to represent the "defective allele" that is a partially deleted version of the dystrophin (DMD) gene. Xylene cyanole (XC) travels more slowly in the gel, and so it will be used to represent the "normal" dystrophin allele. Prepare stock solutions as follows:
   - Label two small beakers or flasks BB and XC. Add 10 ml of deionized water and 1 ml of glycerol to each. Swirl to mix.

   Safety Note: Certain dyes can be dangerous to your health when they are in the dry state but become harmless once they have been dissolved. So weigh out all dyes in a fume hood, and wear a dust mask, goggles, and gloves until they are in solution.

- Weigh out 0.025 grams (25 mg) of bromphenol blue and add it to the BB container.
- Weigh out 0.025 grams (25 mg) of xylene cyanole and add it to the XC container.
- Carefully stir or swirl both containers until the dyes are thoroughly dissolved.

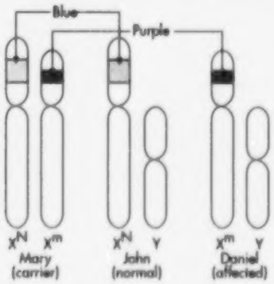
3. **Prepare simulated Smith family DNA samples from the stock dye solutions.** Set up six microcentrifuge tubes, lettered A–F, for each group. Add dyes as shown in the table below. Then cap the tubes and place each set of six in a sandwich baggie or other small container to hand out to each lab group. As you begin the experiment, inform the students which family member is represented by which letter, but do not provide the other information in the table.

Tube	Family member	Genetic condition	Dye sample used
A	Mother	Carrier	25 µl BB & 25 µl XC
B	Father	Normal	50 µl BB
C	Daniel	Affected	50 µl XC
D	Alice	Carrier	25 µl BB & 25 µl XC
E	Michael	Normal	50 µl BB
F	Fetus	Carrier	25 µl BB & 25 µl XC

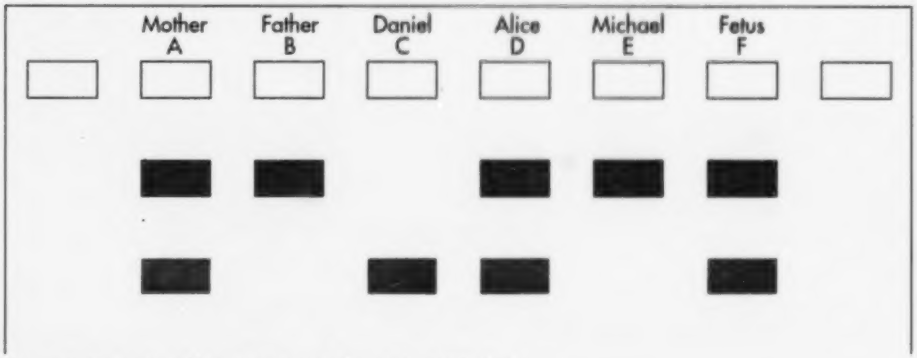
4. **Check that each group of students has established proper electrical polarity before giving them permission to turn on their power supply.** The only way that this experiment can work is if the students electrophorese their dye samples in the correct direction, which is from the black electrode (cathode) toward the red electrode (anode). Emphasize (a) that their gel should be placed in the electrophoresis chamber so that the wells will be at the end with the black electrode, and (b) that they should call you to check things over before they turn on their power supply. If you find a gel in the wrong orientation, do not try to move it, or all of the samples will be lost. Instead, switch the wires at the power supply, so that the black wire is connected to the red terminal and vice versa, thereby ensuring that current coming from the black terminal on the power supply will run to the end of the gel where the samples are located.

ANSWERS TO DMD DIAGNOSIS WORK SHEET STUDENT PAGES 200-201

1. On the diagram below, color the defective alleles purple and the normal alleles blue.


Blue
Purple
X^N X^m
Mary
(carrier)
X^N Y
John
(normal)
X^m Y
Daniel
(affected)

2. On the diagram below
   a. Above each well on this drawing of the gel, put the letter that was on the sample that was loaded into that well.
   b. Using colored pencils, draw all bands that you observed on your gel after electrophoresis.


Mother
A
Father
B
Daniel
C
Alice
D
Michael
E
Fetus
F

3. Complete each of the blanks in the data table below:

Sample	Family Member	# of DMD Alleles	Genotype	Status
Tube A	Mother Mary	2	$X_N X_m$	Carrier
Tube B	Father John	1	$Y X_N$	Healthy
Tube C	Son Daniel	1	$Y X_m$	Has DMD
Tube D	Daughter Alice	2	$X_N X_m$	*Carrier*
Tube E	Son Michael	1	$Y X_N$	*Healthy*
Tube F	Fetus M or [✓ F] (circle)	2	$X_N X_m$	*Carrier*

4. Which allele moves further into the gel, the normal (X^N) or mutant (X^m) allele? Why?
   The mutant (X^m) allele. It has undergone a partial deletion, so it is shorter, and shorter DNA fragments migrate faster than longer ones in gel electrophoresis.

5. Is the daughter, Alice, a carrier for DMD? How can you tell?
   Yes. Her DNA contains two fragments equal in size to the fragments representing the normal and mutant alleles present in her mother's DNA.

6. Does Michael have DMD? How can you tell?
   No. He has only the wild-type (normal) DMD allele.

7. What can you tell the Smith family about their unborn child?
   It is female, and like Mary and Alice, it will be a carrier for DMD.

8. Why are most patients with DMD male?
   A female with DMD is only produced in the extremely rare (1/10,000,000) cases when a carrier female produces an egg bearing the mutant DMD allele and that egg is fertilized by a sperm carrying a brand-new DNA mutation that arose during sperm formation.

9. Can a boy be a carrier for DMD without having the disease? Why or why not?
   No. Boys only have one X chromosome, so if they inherit a mutant DMD allele they get the disease.

10. If you were the genetic counselor in this case, what would you tell the Smiths about their test results?
    That there is some good news and some bad news. The good news is that neither Michael nor the unborn child will get DMD. The bad news is that both Alice and the unborn daughter are carriers.

Career

CHAPTER 3

How Genes and the Environment Influence Our Health

SECTION F

How Can I Become a Genetic Counselor ?

Chapter 3: Section F Background and Overview

EXPERIENCE INDICATES THAT AFTER completing this chapter (and particularly sections D and E), many students want to know what is involved in preparing for a career as a genetic counselor. This section is intended to be optional reading material for such students.